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Patterns of Growth and Development in Pleurocapsalean Cyanobacteria

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INTRODUCTION

Cyanobacteria belonging to the two orders *Chamaesiphonales* and *Pleurocapsales* are sessile organisms that reproduce by the formation of small, spherical cells known as spores. This mode of reproduction distinguishes them from all other cyanobacteria.

The members of the order *Chamaesiphonales* are unicellular and fall into two subgroups, exemplified by the genera *Chamaesiphon* and *Dermocarpa*, which differ in their mode of spore formation. The spores of *Chamaesiphon* (termed "exospores") are formed by successive cleavages from the apical pole of a sessile, elongated vegetative cell. A study of the development of two *Chamaesiphon* strains in pure culture has shown that they are budding organisms with a life cycle analogous to that of *Nitrobacter winogradskyi* and certain *Rhodopseudomonas* species *R. palustris, R. viridis,* and *R. acidophila*) (41). The exospore is the smaller daughter

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cell formed through this variant of binary fission.

The spores of *Dermocarpa* (termed "endospores") arise through a process of multiple fission. The spherical vegetative cell, formed through the enlargment of an endospore, cleaves into a large number of small daughter cells. After cleavage is complete, the daughter cells, or endospores, are released through rupture of the parental wall; each subsequently develops into a vegetative cell.

All members of the order *Pleurocapsales* reproduce by multiple fission and endospore release. The only property that distinguishes them from endospore-forming members of the *Chamaesiphonales*, such as *Dermocarpa*, is their ability to divide by binary, as well as by multiple, fission. The initial enlargement of the endospore is followed by repeated binary fissions, producing a coherent mass or aggregate of vegetative cells, some or all of which then undergo multiple fission and release endospores.

For reasons that will be fully explained in a later section of this review, we propose to place unicellular organisms, such as *Dermocarpa*, that reproduce by endospore formation in the order *Pleurocapsales*. We shall therefore use the ordinal name "*Pleurocapsales*" and the common name "pleurocapsalean cyanobacteria" to designate all cyanobacteria that reproduce by the liberation of endospores formed through multiple fission.

Pleurocapsalean cyanobacteria have a wide natural distribution, occurring in terrestrial, freshwater, and marine environments. The intertidal zone contains a particularly abundant and diverse population of these organisms, which grow as epiphytes on other algae and as epiliths on rocks and the shells of marine invertebrates (3, 14, 34). Some are endoliths and possess the remarkable property of penetrating into calcareous substrates, where they develop in microscopic tunnels formed through dissolution of calcite (11, 19, 27).

Prior to the work that will be described here, very few pleurocapsalean cyanobacteria had been cultivated (2, 4, 23, 28, 30), and only four strains had been isolated in the axenic state (see Tables 2 and 3). The existing accounts of their structure and development are, therefore, almost entirely based on microscopic examination of materials collected in the field. During the past 5 years we have isolated axenic cultures of many marine pleurocapsalean cyanobacteria and have purified a number of impure freshwater strains received from other investigators. As judged from existing taxonomic descriptions (3, 6, 14, 34), this collection is reasonably representative of the internal diversity of the whole group of pleurocapsalean cyanobacteria. We have thus been able to make a comparative study of pleurocapsalean structure and development under defined growth conditions. The following account of the pleurocapsalean cyanobacteria is largely based on our own observations, but we will attempt to evaluate findings of other workers where relevant.

The small, spherical reproductive cell termed an endospore by phycologists differs from the bacterial endospore in its mode of formation, structure, and development. In a procaryotic context, such a dual use of the term is undesirable, and it is therefore necessary to find another designation for the pleurocapsalean endospore. One possibility is *nannocyte*, a term that has been used to describe unusually small cells produced by cyanobacteria (3, 13, 14). However, it has never been precisely defined, and it has been applied to small cells in cyanobacterial groups that never form endospores, notably, the *Chroococcales* (13–16). To redefine it now as a substitute for endospore would, therefore, almost certainly cause confusion. A new name for the pleurocapsalean endospore must, accordingly, be found. We shall call this cell a *baeocyte* (Greek, "small cell").

(This monograph is based on the dissertation of J.B.W. submitted in partial fulfillment of the requirements for the Ph.D. degree at the University of California, Berkeley.)

METHODS FOR STUDY OF GROWTH AND DEVELOPMENT

Principal Media and Growth Conditions

Three basic media, the compositions of which are shown in Table 1, were used routinely during the course of this study. Medium BG-11 (39) was used as the standard medium for strains of nonmarine origin. Medium MN, used as the standard medium for marine strains, has a seawater base and is fortified with the minerals of medium BG-11. Medium ASN-III is a strictly synthetic medium suitable for growth of marine strains. Merck analytical grade chemicals and deionized water, passed through a Millipore Super-Q water purifier, were used for the preparation of all media. Solid media contained Difco agar at a final concentration of 1.2% (wt/vol). The mineral base and the agar solution were separately autoclaved (1) at the required concentrations and mixed just before pouring the plates.

Two strains (7301 and 7317) have an obligate requirement for vitamin B_{12}. For the culture of these strains, vitamin B_{12} was added to media after autoclaving at a final concentration of 4 μg/liter.

The media described above are poorly

TABLE 1. *Compositions of standard media*

Medium	Ingredient (per liter): Deionized water (ml)	Seawater (ml)	NaCl (g)	$MgSO_4 \cdot 7H_2O$ (g)	$MgCl_2 \cdot 6H_2O$ (g)	KCl (g)	$CaCl_2 \cdot 2H_2O$ (g)	$NaNO_3$ (g)	$K_2HPO_4 \cdot 3H_2O$ (g)	Citric acid (g)	Ferric ammonium citrate (g)	EDTA[b] (g)	Na_2CO_3 (g)	Trace metals A-5[c] (ml)	pH after autoclaving	Osmolarity[a] (milliosmoles)
BG-11	1,000			0.075			0.036	1.5	0.04	0.006	0.006	0.001	0.02	1.0	7.1	
MN	250	750		0.04			0.02	0.75	0.02	0.003	0.003	0.0005	0.02	1.0	8.5	690
ASN-III	1,000		25.0	3.5	2.0	0.5	0.5	0.75	0.02	0.003	0.003	0.0005	0.02	1.0	7.3	825

[a] Determined by freezing point depression.

[b] EDTA, Ethylenediaminetetraacetate (disodium potassium salt).

[c] A-5 trace metal mix consisted of (grams per liter): H_3BO_3, 2.86; $MnCl_2 \cdot 4H_2O$, 1.81; $ZnSO_4 \cdot 7H_2O$, 0.222; $Na_2MoO_4 \cdot 2H_2O$, 0.039; $CuSO_4 \cdot 5H_2O$, 0.079; $Co(NO_3)_2 \cdot 6H_2O$, 0.0494.

buffered. For some experiments, buffering capacity was increased by addition of tris-(hydroxymethyl)aminomethane - hydrochloride buffer (pH 7.5), at a final concentration of 0.1% (wt/vol), to media after autoclaving. However, at this concentration several strains (notably, 7301, 7302, 7303, 7304, 7305, and 7306) were inhibited by tris(hydroxymethyl)aminomethane buffer.

Illumination of cultures was continuous and was provided by fluorescent light sources. Osram Interna fluorescent lamps were used to illuminate liquid cultures of small volume and plate cultures. Most of the organisms studied do not tolerate high light intensities. Stock cultures and plate cultures were generally exposed to light intensities of between 200 and 400 lx, measured at the culture surfaces.

No attempt was made to determine temperature optima for the strains examined. With one exception, all strains grew well at 25 to 28°C, which was routinely used for their cultivation. One strain of thermophilic origin (7327) failed to grow within this temperature range and was routinely grown at 37°C.

Stock cultures were maintained in 100-ml Erlenmeyer flasks containing 50 ml of liquid medium and incubated without agitation or aeration.

Cooper Dish Culture Technique

Pleurocapsalean cyanobacteria grow very slowly: weeks may be required for completion of their development. Thus, it was necessary to find a method of culture that would sustain growth and permit semicontinuous observations of development over long periods. Cooper culture dishes (Falcon, no. 3009), which were originally designed for the examination of liquid tissue cultures, could be satisfactorily adapted for this purpose. A liquid agar medium (8.0 ml) was pipetted into the lower half of the dish, completely filling the space below the depressed surface of the lid. After the medium had hardened, the lid was rotated and detached from the underlying agar, and the agar surface was inoculated with a suitable dilution of freshly released baeocytes. The lid was then replaced, and the dish was incubated at 25°C in a small clear plastic box that contained tissue paper moistened with a copper sulfate solution to retard evaporation and prevent fungal growth. These cultures were illuminated from above with light at intensities between 200 and 300 lx. At intervals, the cultures were placed on a microscope stage and examined. Photographs were made with a Zeiss Universal microscope equipped with a long focal-length condenser and a ×16 Neofluar objective, using bright-field illumination.

The above system has optical limitations, imposed by both the thickness of the agar layer and the relatively low power of the objective. However, it permits good growth over long periods (up to 4 weeks) without risk of contamination and has proved extremely useful for determining cyanobacterial developmental cycles.

It should be noted that no attempt was made to incubate Cooper dish cultures under conditions that permitted growth of each strain at the maximal rate. For example, light intensities were always kept low, because experience showed that freshly liberated baeocytes are often very sensitive to light. For this reason, the relative growth rates revealed by time lapse photographs of developmental sequences are not strictly comparable with one another and in many cases are far below the growth rates obtainable in liquid cultures at optimal light intensities and temperatures.

Baeocyte Motility and Baeocyte Counts

To demonstrate the presence or absence of baeocyte motility, freshly released baeocytes were placed in a light gradient; motile baeocytes display a phototactic response under these conditions, whereas immotile baeocytes do not.

An agar plate was spread with a drop of a suspension from a liquid culture in the stationary phase and subsequently incubated in a clear plastic box illuminated laterally. The light intensity at the brightly illuminated side of the box ranged from 50 to 200 lx. After baeocyte release, the location of the baeocytes relative to the mother cell from which they had arisen was determined by periodic microscopic examination. It is sometimes necessary to test several different light intensities and growth media to demonstrate unambiguously the occurrence of a phototactic response.

The numbers of baeocytes released by single parental cells of *Dermocarpa* and *Dermocarpella* strains were determined in Cooper dish cultures subjected to unilateral illumination, to provoke phototactic baeocyte dispersal. The baeocyte populations derived from single parental cells were photographed, and baeocyte counts were made from prints.

Microscopy

Unless otherwise noted, all light micrographs were taken with bright-field illumination, using a Zeiss Universal microscope equipped with Neofluar objectives.

Most fixations for electron microscopy were done on cells grown in liquid cultures or on agar plate surfaces. Fixations of undisturbed colonies were made on cultures grown in 0.5% agar overlays on plates of solid media. To observe cell attachment, cultures were grown on the surfaces of small squares of dialysis membrane that were placed on the surface of agar plates.

The fixation, dehydration, and embedding procedures for electron microscopy have been previously described by Rippka et al. (33). In general, cells were prefixed (0.5%) and fixed (3.5%) in glutaraldehyde, with subsequent postfixation in osmium (1.0%). During fixation, marine strains were buffered in 0.143 M Veronal-acetate buffer at pH 7.2 supplemented with $CaCl_2 \cdot 2H_2O$ (1.0 g/liter)-sucrose (34.0 g/liter)-NaCl (10.0 g/liter). Nonmarine strains were buffered in 0.143 M Veronal-acetate buffer at pH 7.2 supplemented with $CaCl_2 \cdot 2H_2O$ (1.0 g/liter).

The initial steps of the fixation procedures varied with the growth conditions used. Cells grown in liquid media were centrifuged at low speed before prefixation, whereas cells grown on solid media could be scraped off the agar surface and suspended in prefixative directly. In both cases, the cells were enrobed in agar after fixation in glutaraldehyde. Entire undisturbed colonies, grown in agar overlays, were removed by cutting small blocks out of the overlay and dropping them directly into prefixative, which made it unnecessary to enrobe them subsequently in agar. Small squares of dialysis membrane with cells attached to their upper surfaces were removed from agar plate surfaces and dropped directly into prefixative. The cells remained attached to the membrane throughout the entire fixation, dehydration, and embedding procedure. With the exception of the squares of dialysis membrane, which required flat embedding, all preparations were embedded in Beem capsules.

ISOLATION AND PURIFICATION

The nutritional requirements of members of the *Pleurocapsales* do not differ significantly from those of other cyanobacteria that occur in the same natural habitats. However, their rates of growth are, in general, low. Consequently, to prevent overgrowth by other cyanobacteria, primary cultures are best established by direct isolation from favorable source materials on solid media, rather than by preliminary cultivation in a liquid medium. Rock chips, mollusk shells, and macroalgae from the intertidal zone served as sources of marine representatives. During transport to the laboratory, samples were kept in closed bottles or tubes that each contained a piece of damp filter paper. Complete immersion in seawater should be avoided, since this often leads to rapid growth of non-photosynthetic bacteria.

As soon as possible after collection, the samples were examined microscopically to determine the most appropriate isolation procedure. Material was scraped off the natural substrate and suspended in sterile liquid medium, tough aggregates being dispersed in a tissue homogenizer or by grinding between two flame-sterilized microscope slides. When microscopic examination revealed the presence of a large number of contaminating organisms, the suspension was washed several times in sterile medium by repeated low-speed centrifugations. This treatment substantially reduced the population of contaminants.

After such preliminary treatment, the sample was streaked on several plates and also inoculated into a tube of liquid medium. Since well-isolated colonies are relatively rare on primary plates, the probability of a successful isolation is increased if several plates are prepared from each sample. Members of the *Pleurocapsales* develop on plates as small, compact colonies, which are often tough and adhere to the agar surface. Early detection and transfer of such colonies is essential, to minimize the possibility of overgrowth by other organisms. Consequently, plate cultures were examined daily with a dissecting microscope, and microcolonies were transferred as soon as they could be recognized. If such colonies could be easily dispersed, they were suspended in sterile medium and restreaked. However, in many cases it is preferable to pick the entire colony off the plate with a platinum spade under the dissecting microscope and transfer it to a tube of liquid medium, from which fresh plate cultures can be prepared after sufficient growth has occurred. When primary plates did not yield any well-isolated colonies, the primary liquid culture was used to prepare a second set of plates. In all cases, pure cultures were obtained by repeated plating.

With the precautions described above, the primary isolation and subsequent purification of members of the *Pleurocapsales*, as well as other cyanobacteria that form discrete colonies, can be readily achieved by standard bacteriological procedures. All our own primary isolates were derived from marine inocula. However, the same procedures were used to obtain axenic cultures of freshwater strains received impure from other investigators, which were in many cases heavily contaminated with non-photosynthetic bacteria and fungi at the time of receipt.

Cultures were presumed to be axenic when plates showed only one type of colony. This was confirmed by microscopic examination of liquid cultures and by the absence of growth in a mineral medium supplemented with glucose (0.3%, wt/vol)-yeast extract (0.05%, wt/vol) and incubated in the dark.

STRAIN HISTORIES

The sources and histories of the 32 axenic strains used in this study are summarized in Tables 2 (marine isolates) and 3 (terrestrial and freshwater isolates). The symbols P (pure) and I (impure) indicate the state of strains at the time of accession from other investigators and from culture collections.

GENERAL ASPECTS OF STRUCTURE AND DEVELOPMENT

Difference Between Multiple Fission and Binary Fission

The ability to undergo cell division by multiple fission is a fundamental property of the *Pleurocapsales* and distinguishes them from all other cyanobacteria. Multiple fission can be defined as the rapid division of a vegetative cell into at least four (and often many more) spherical daughter cells. No significant increase in cell volume occurs during the course of multiple fission, and, consequently, the volume of each daughter cell is a quarter or less than that of the parental cell. This marked diminution in cell size that accompanies multiple fission distinguishes it from a series of binary fissions, in which each round of division is followed by cell growth, with the result that the volume of each daughter cell is never less than half that of the parental cell.

Most existing reports on multiple fission in the *Pleurocapsales* indicate that it takes place by successive cleavages (6, 13, 16); however, Setchell and Gardner (34) claimed the occurrence of simultaneous cleavage in *Dermocarpa fucicola*. This specific claim of Setchell and Gardner could not be verified in the present investigation. As will be described later, multiple fission in the *Pleurocapsales* always takes place by rapid, successive cleavages. However, in some members of the group (including *Dermocarpa*) this cannot be ascertained by light microscopy and can be established only through the examination by electron microscopy of thin sections of dividing cells.

Roles of Multiple Fission and Binary Fission in Development

After completion of multiple fission, the daughter cells, or baeocytes, are released by rupture of the parental cell wall. Baeocyte formation is the sole means of reproduction in all members of the *Pleurocapsales*.

In unicellular members of the *Pleurocapsales* (*Dermocarpa* and *Xenococcus*), cell division oc-

TABLE 2. *Marine isolates*[a]

Strain no.	Name (if any) previously applied	Source and date collected	Previous history (if any)	ATCC no.
7301	*Dermocarpa violacea*[b]	Marine aquarium, Scripps Institute of Oceanography, La Jolla, Calif., Dec. 1964	Isolated by R. Lewin CCCAP (strain 1416/1) (P), 1965 PCC (P), 1972	29367
7302		Seawater tank, Arizona Marine Station, Puerto Penasco, Mexico, Mar. 1971		29368
7303		Same as for 7302		29369
7304		Epiphyte on *Rhodochorton* sp., high intertidal zone, Bodega Marine Laboratory, Calif., Jan. 1970		29370
7305	*Dermocarpa* sp.[b]	Marine aquarium, Scripps Institute of Oceanography, La Jolla, Calif.	Isolated by R. Lewin PCC (P), 1971	29373
7306		Rock chip, high intertidal zone, Pillar Point, Calif., Feb. 1970		29374
7307		Rock chip, high intertidal zone, Horseshoe Cove, Bodega Marine Laboratory, Calif., Jan. 1970		29375
7310		Snail shell, intertidal zone, Arizona Marine Station, Puerto Penasco, Mexico, Mar. 1971		29385
7312		Same as for 7310		29377
7314		Same as for 7310		29386
7317		Same as for 7310		29387
7319		Same as for 7310		29388
7320		Same as for 7310		29389
7321		Same as for 7310		29390
7322		Same as for 7310		29391
7324		Same as for 7310		29392
7325		Same as for 7310		29378
7326		Same as for 7310		29376
7440		Rock chip, high intertidal zone, Deuville, Normandy, France, Mar. 1974		29394
7506		Rock chip, collected by T. LeCampion-Alsumard, Marseille, France, Sept. 1974		29395
7516	*Hyella caespitosa*[c]	Rock chip, Station B, l'Ile Riou, Calanque des Contrebandiers, Marseille, France, 1974	Isolated by T. LeCampion-Alsumard PCC (I), Apr. 1975	29396 (ref. 27, 28)

[a] Abbreviations: PCC, Paris Culture Collection; CCCAP, Cambridge Culture Center for Algae and Protozoa; ATCC, American Type Culture Collection; I, impure when received from indicated source; P, pure when received from indicated source.

[b] Identified by R. Lewin.

[c] Identified by T. LeCampion-Alsumard.

curs only by multiple fission. After release, the baeocyte develops into a large, spherical vegetative cell, which again undergoes multiple fission. In other members of the group, however, baeocyte enlargement is followed by binary fission, to produce an aggregate of vegetative cells. In *Dermocarpella*, only one to three binary fissions intervene before the onset of multiple fission. In other genera, binary fission is more extensive, the gross form of the aggregate produced being determined by the planes of successive binary fissions. Regular binary fission in three planes at right angles to one another produces cubical packets of cells, characteristic of *Myxosarcina* and *Chroococcidiopsis*. In the *Pleurocapsa* group, the planes of successive binary fissions are less regular, and the aggregate consists of a mass of vegetative cells, sometimes bearing filamentous extensions, either branched or unbranched. Some or all of the cells that compose such aggregates eventually undergo multiple fission and release baeocytes.

Structure of the Cell Wall: PG, OM, and F Layers

Although multiple fission leading to baeocyte formation is the primary character that differentiates the *Pleurocapsales* from other sub-

TABLE 3. *Terrestrial and freshwater isolates*[a]

Strain no.	Name (if any) previously applied	Source and date collected	Previous history (if any)	ATCC no.	Ref.
6712	*Chlorogloea* sp.	Water sample, reservoir, Marin County, Calif., 1967	Isolated by R. Kunisawa	27176	39
7203	*Myxosarcina chroococcoides*[b]	Soil sample, near Greifswald, E. Germany, 1962	EMAU (strain A149), 1962		
	Chroococcidiopsis thermalis[c]		CCCAP (strain 1451/1) (I), 1965	27900	21, 23
			PCC (P), 1972		
7327	*Pleurocapsa minor*	Hunter's Spring, Oregon, 1969	Isolated by R. Castenholz (strain OH-69-PM)		
			PCC (I), 1973	29393	4
7431	*Chroococcidiopsis thermalis*[b]	Mineral spring, near San Diego, Cuba, 1964	Isolated by J. Komárek (strain 1964/48)		
			PCC (I), 1974	29379	21, 23
7432	*C. thermalis*[b]	Mineral spring, near Santa Fe, Cuba, 1965	Isolated by F. Hindák (strain 1965/21)		
			PCC (I), 1974	29380	21, 23
7433	*C. thermalis*[b]	Soil, dried pool, Pinar del Rio, Cuba, 1966	Isolated by F. Hindák (strain 1966/27)		
			PCC (I), 1974	29381	21, 23
7434	*Chroococcidiopsis cubana*[b]	Pool, botanical garden, Havana, Cuba, 1965	Isolated by F. Hindák (strain 1965/19)		
			PCC (I), 1974	29382	21, 23
7436	*C. cubana*[b]	Drainage ditch, Nueva Gerona, Cuba, 1965	Isolated by F. Hindák (strain 1965/108)		
			PCC (I), 1974	29382	21, 23
7437	*Chroococcidiopsis cyanosphaera*[b]	Same as for 7434	Isolated by F. Hindák (strain 1965/25)		
			PCC (I), 1974	29371	21, 22
7438	*C. cyanosphaera*[b]	Same as for 7432	Isolated by F. Hindák (strain 1965/26)		
			PCC (I), 1974	29372	21, 22
7439	*Chroococcidiopsis doonensis*[b]	Sand, sea beach, near Mamaia, Romania, 1968	Isolated by F. Hindák (strain 1968/64)		
			PCC (I), 1974	29384	21, 23

[a] Abbreviations: PCC, Paris Culture Collection; CCCAP, Cambridge Culture Center for Algae and Protozoa; EMAU, Ernst Moritz Arndt University; ATCC, American Type Culture Collection; I, impure when received from indicated source; P, pure when received from indicated source.

[b] Identified by J. Komárek.

[c] Subsequent identification by J. Komárek.

groups of cyanobacteria, these organisms share other distinctive common structural properties, notably, the special structure of the cell wall. The cell walls of cyanobacteria, like those of other gram-negative procaryotes, are always composed of at least two layers that differ both in fine structure and in chemical composition. The inner wall layer appears homogeneous and electron dense in thin sections and is largely (perhaps entirely) composed of peptidoglycan (PG layer) (7, 9, 10, 20). It is surrounded by a somewhat thicker layer of lower electron density, which has the typical fine structure of a unit membrane and has been shown, in several cyanobacteria, to contain lipopolysaccharides (42). It thus corresponds to the so-called outer membrane layer of other gram-negative procaryotes (OM layer).

In the *Pleurocapsales*, the wall of the vegetative cell is always surrounded by a third, external layer, which has a fibrous structure as visualized in electron micrographs of thin sections (F layer) (Fig. 1). Although it resembles in fine structure the tubular sheath that encloses the trichome of certain oscillatorian cyanobacteria (24), the F layer in the *Pleurocapsales* is always closely appressed to the outer surface of the OM layer and is, thus, rarely distinguishable by light microscopy as a separate structure.

During the enlargement of a pleurocapsalean vegetative cell, the widths of the PG and OM layers remain constant, being approximately 6 and 7.5 nm, respectively, as measured on electron micrographs of thin sections. In contrast, the thickness of the F layer increases continuously throughout the enlargement of the cell

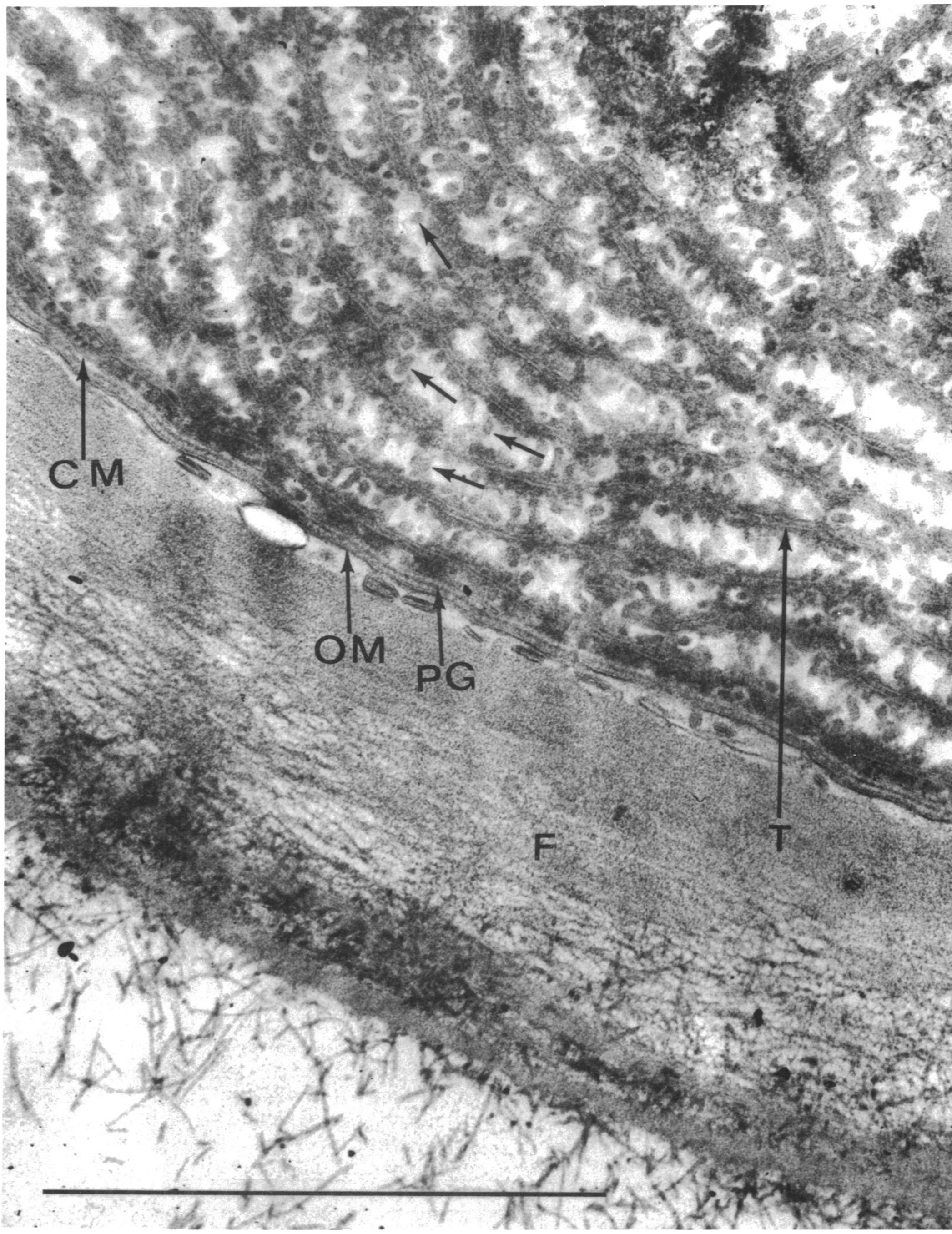

FIG. 1. *Electron micrograph of a thin section of part of a large vegetative cell of Dermocarpa (7302). F, Fibrous outer wall layer, 750 nm thick and of variable density; OM, outer membrane wall layer; PG, peptidoglycan wall layer; CM, cytoplasmic membrane; T, thylakoids. Glycogen granules are indicated by arrows. Bar = 1.0 μm in all electron micrographs.*

that it encloses. In *Dermocarpa*, where the vegetative cell can attain a diameter of 30 μm before the onset of multiple fission, the surrounding F layer may be over 1,000 nm thick (Fig. 1).

Role of F Layer During Binary Fission

In pleurocapsalean cyanobacteria that undergo binary fission during their development, the synthesis of the three wall layers during cell division follows a well-defined and characteristic course. The transverse wall is formed through ingrowth first of the PG layer and subsequent ingrowth of the OM layer; the F layer does not participate (Fig. 2). After delamination of the transverse wall, each daughter cell, now completely enclosed by the PG and OM layers, begins to synthesize a new F layer, which is laid down evenly over the entire surface of the OM layer. Daughter cells are, consequently, pushed apart from one another by the intercalation of new F-layer material but remain enclosed by the common F layer synthesized by the parental cell. The discontinuous formation of the F layer, which begins anew at each succeeding cell generation, is particularly evident in electron micrographs of thin sections of members of the *Pleurocapsa* group, where several distinct F layers surround the cells that compose the aggregate (Fig. 3). The material of the F layer serves as a semielastic integument, which firmly binds together the vegetative cells produced through successive binary fissions and maintains them in a fixed topological relationship. Since the elasticity of the F layer is limited, the oldest, external F layer enclosing a cell aggregate becomes frayed and irregular and eventually tears open as it is progressively stretched by the increase of the volume of cells that it encloses. However, even very large aggregates of vegetative cells remain coherent, presumably through adhesion between contiguous F layers (Fig. 2).

Even in pleurocapsalean cyanobacteria, such as *Pleurocapsa*, that produce large and partly filamentous aggregates, the vegetative cells comprising the aggregate are all physically separated from one another by interposed F-layer material. However, this is not necessarily evident when such aggregates are examined by light microscopy, since the F layers between adjacent vegetative cells may not be sufficiently thick to permit their resolution. Sometimes, the F layer can be perceived as a transparent intercellular gap.

Role of F Layer During Multiple Fission and Baeocyte Release

The synthesis of wall material during multiple fission does not occur in the same manner in all pleurocapsalean cyanobacteria. In two genera, *Xenococcus* and *Chroococcidiopsis*, the cleavage products formed at each successive division, enclosed by the PG and OM layers, immediately begin to synthesize a new F layer. Consequently, when multiple fission is completed, each baeocyte is enclosed by a PG layer, an OM layer, and a thin F layer. Wall synthesis accompanying multiple fission in these two groups is identical to that which accompanies binary fission; the baeocytes differ from vegetative cells only in size, not in wall structure.

In the genera *Dermocarpa*, *Dermocarpella*, and *Myxosarcina* and the *Pleurocapsa* group, the synthesis of new F-layer material is completely arrested throughout multiple fission, with the result that each baeocyte is enclosed only by the PG and OM layers of the cell wall. Light microscopy does not reveal successive division stages of multiple fission in such organisms because of the relative thinness and weak refractility of the transverse walls.

The release of baeocytes from parental cells has been attributed by earlier workers either to rupture or to "gelatinization" of the enclosing wall. Our observations show that it always takes place through a localized break or tear in the fabric of the enclosing parental F layer. This is particularly evident in *Dermocarpa* (Fig. 4), as a result of the large size of the vegetative cells and the consequent thickness of their F layers. The sharpness of the break in the F layer suggests that rupture results from stress, rather than from enzymatic dissolution of the F-layer material.

Movement of Baeocytes and Its Relation to Wall Structure

Vegetative cells of pleurocapsalean cyanobacteria are completely immotile. However, there are two brief reports that baeocytes of *Pleurocapsa fuliginosa* (2) and *Endonema gracile* (31) display jerking movements after their release.

We have observed that in all genera where synthesis of the F layer is arrested during multiple fission, the baeocytes are capable of gliding movement after release. The ability to glide is transitory, being lost after some hours, probably when synthesis of a new F layer begins. Gliding movement is most clearly manifested when freshly released baeocytes are placed in a light gradient, which elicits a phototactic response. The baeocytes may move either toward or away from the light source, depending on its intensity. Figure 5 shows the behavior in a light gradient of the population of baeocytes released on an agar plate from one parental cell of *Dermocarpa* and photographed at intervals over a period of 25 h. Comparison of successive fields shows that,

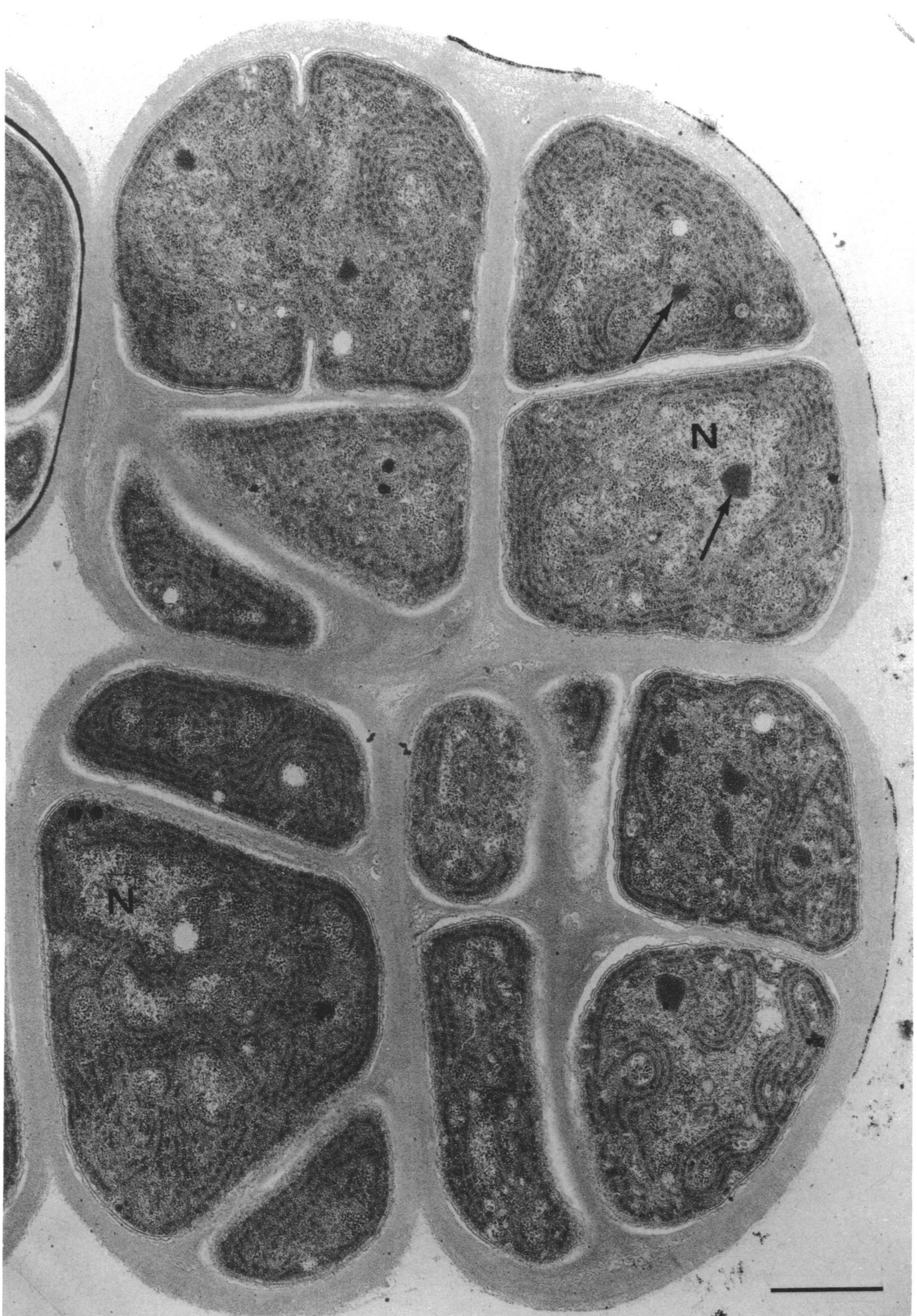

FIG. 2. *Electron micrograph of a thin section of Chroococcidiopsis strain 7432 grown in an agar overlay of medium BG-11. See text for discussion. Carboxysomes are indicated by arrows and nucleoplasm is indicated by N.*

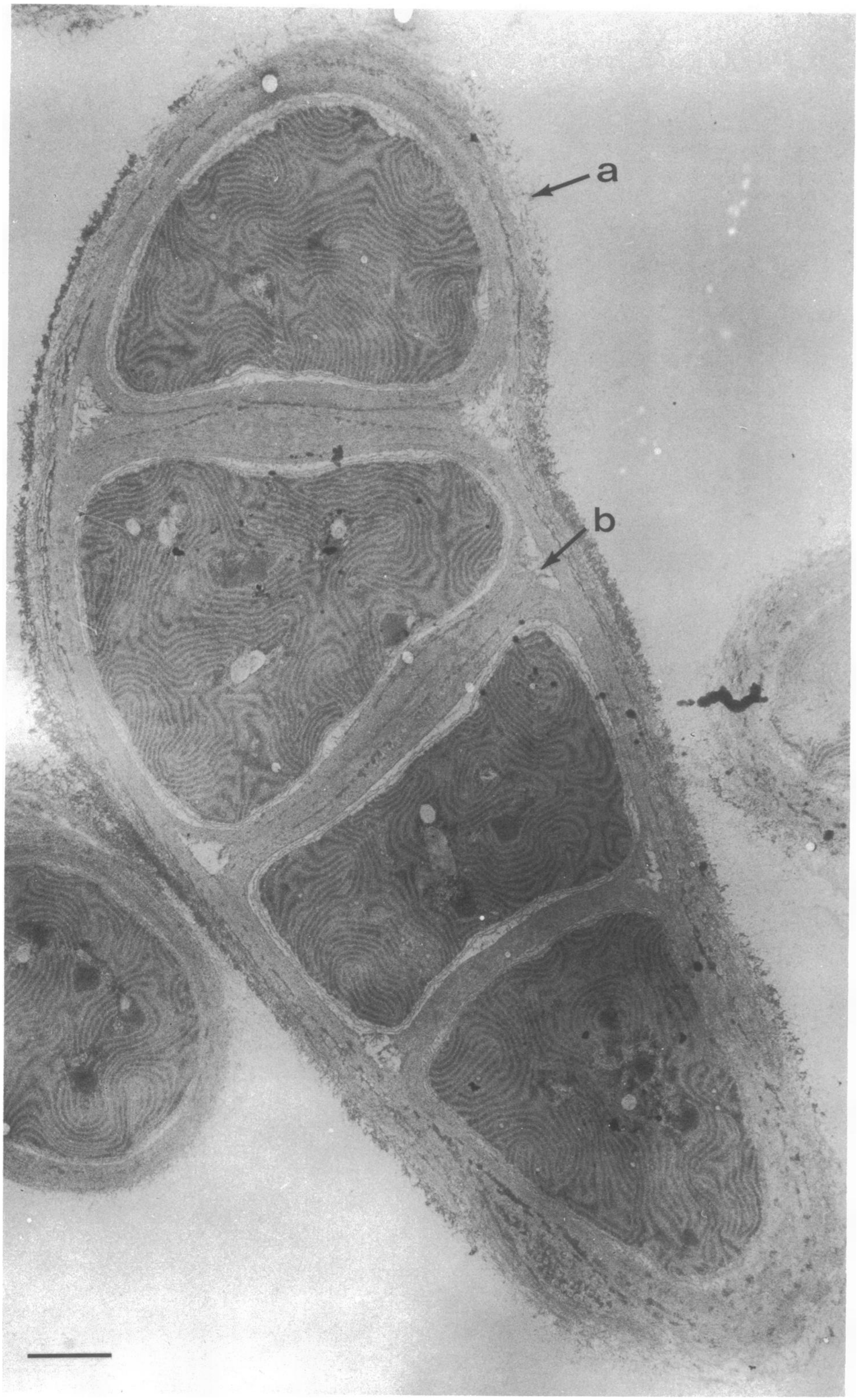

FIG. 3. *Electron micrograph of a thin section of a member of the Pleuroscapsa group (7314) grown in an agar overlay on medium MN. Each cell is enclosed by individual PG, OM, and F wall layers. The group of cells is also enclosed by additional F layers, synthesized during previous generations. As cell volume increases, the more external layers of F wall material become either stretched (a) or torn (b).*

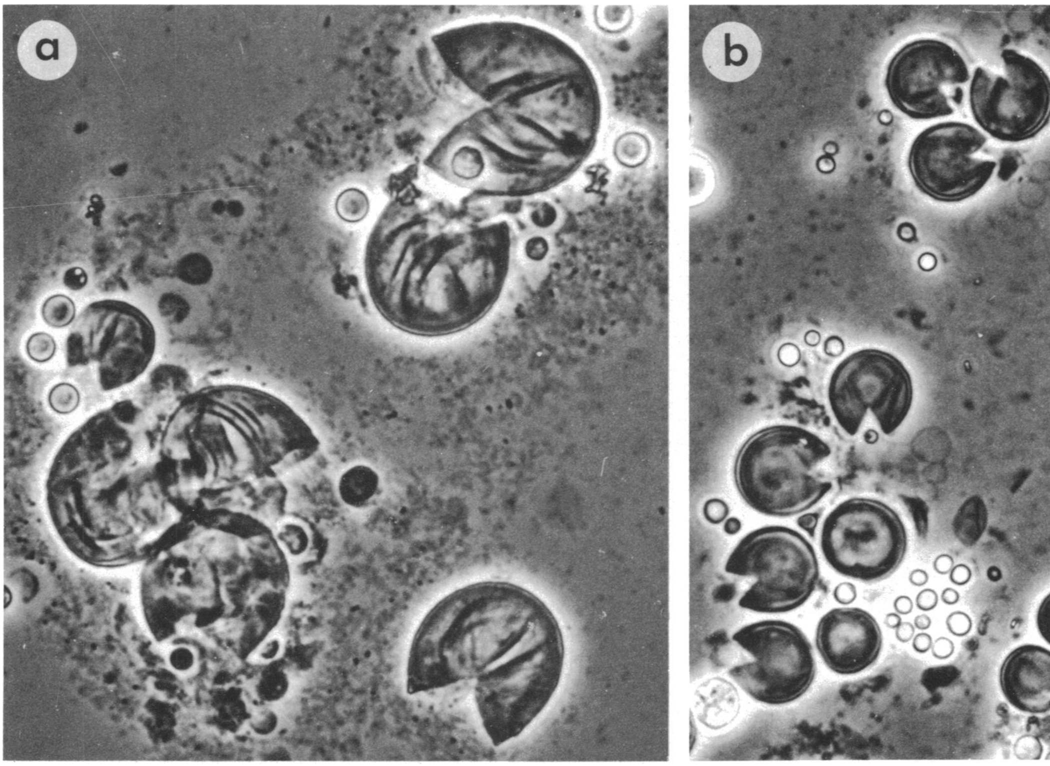

FIG. 4. *F layers of parental cells, emptied of their baeocytes, in aging liquid cultures of Dermocarpa. (a) Strain 7437; (b) strain 7304. Phase contrast, ×1,000.*

initially, nearly all the baeocytes are motile. A progressive and apparently random loss of motility thereafter occurs in the population: some baeocytes become stationary after 6 h or less, and a few remain motile for as long as 48 h. In this strain (7302), the rate of movement of the baeocytes is approximately 120 μm/h at 22°C.

The baeocytes of *Xenococcus* and *Chroococcidiopsis*, which have already synthesized an F layer at the time of their release, have never been observed to glide. Even in a light gradient, they remain closely clustered around the parental cell after release (Fig. 6).

These observations suggest that expression of the capacity for gliding movement is prevented when a pleurocapsalean cell is enclosed by an F layer. In other words, the mechanism of gliding movement can operate only if the OM layer of the wall is in direct contact with the substrate.

Attachment of Baeocytes

Pleurocapsalean cyanobacteria characteristically occur attached to solid substrates in natural environments. This property is also displayed in axenic cultures. In unagitated liquid media, the growing population is firmly attached to the glass walls of the culture vessel. If the vegetative cells (or cell aggregates) are detached from the glass surface by vigorous agitation, they do not readhere, remaining suspended in the medium. However, when baeocyte release occurs, the baeocytes rapidly become attached to the wall of the culture vessel. Baeocytes released on the surface of an agar plate stick to the agar and are difficult to detach. These observations suggest that adhesiveness is a property of baeocytes, not of vegetative cells, but the mechanism of baeocyte adhesion is unknown. Electron microscopic examination of thin sections has failed to reveal specific attachment organelles, comparable to the caulobacter holdfast (32).

Although attachment seems to occur at the baeocyte stage, its firmness may increase during subsequent vegetative growth, as a result of F-layer synthesis. This is suggested by electron micrographs of thin sections of *Dermocarpella* grown on a piece of cellophane placed on the surface of an agar plate (Fig. 7). The F layer flares out around the base of the developing vegetative cell, thus enlarging considerably the area of contact with the substrate.

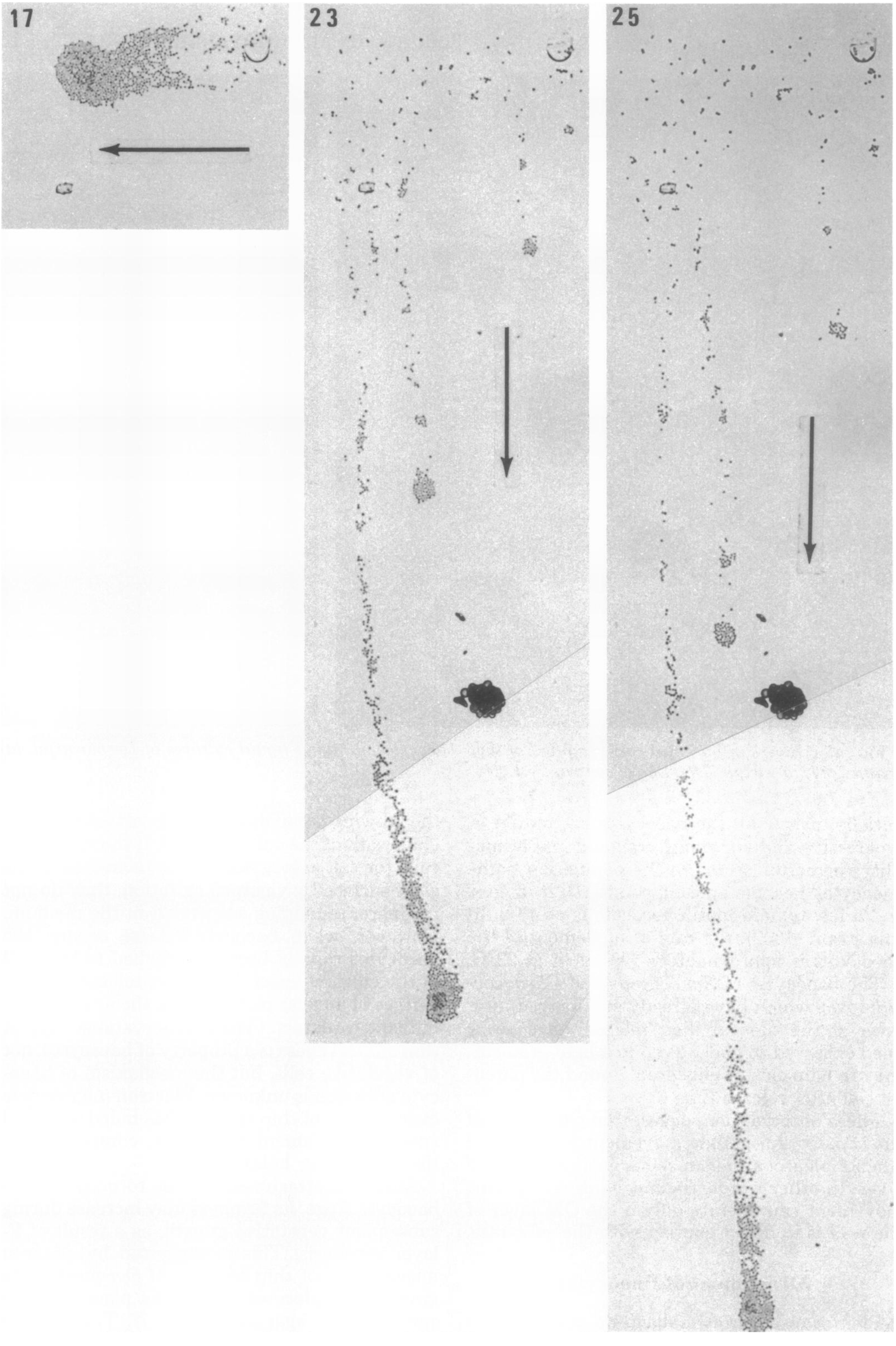

FIG. 5. *Phototactic response of baeocytes liberated from a parental cell of Dermocarpa (7302) that had been placed in a Cooper dish culture on medium MN and illuminated laterally with unidirectional light. Successive light micrographs of the same field, taken 17, 23, and 25 h after the preparation was made. Arrows indicate the direction of illumination, which was changed at 17 h. Note the remnants of the parental F layer in the upper right-hand corner of each picture. ×200.*

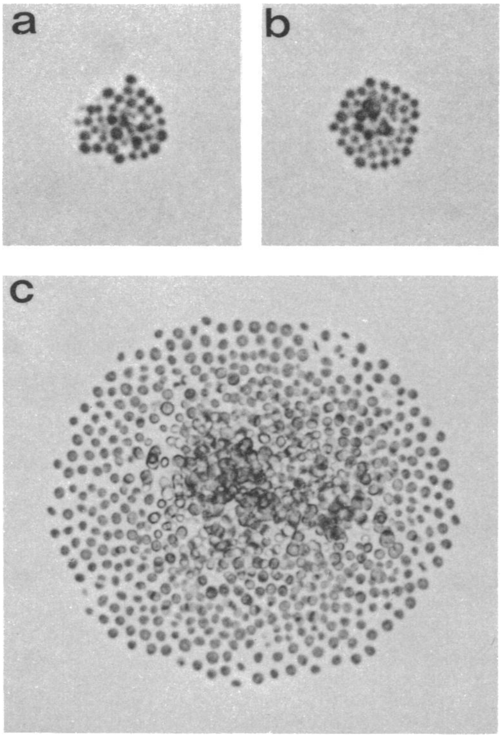

FIG. 6. *Light micrographs of groups of baeocytes after release in Cooper dish cultures subjected to unidirectional illumination. (a and b) Xenococcus (7305), ×470; (c) Chroococcidiopsis (6712), ×280. The immotile baeocytes do not show a phototactic response and remain closely and evenly clustered around the remains of the parental cells.*

Internal Fine Structure of the Cell

The internal fine structure of the pleurocapsalean cell is, in general, similar to that of other cyanobacterial cells (25); no features that could be considered distinctive of this group have been observed. The cytoplasmic membrane closely follows the contour of the cell wall and is devoid of intrusions (Fig. 1). The thylakoids, 17.5 nm in width and composed of two closely appressed unit membranes, are dispersed throughout the cytoplasm in somewhat irregular parallel groups (Fig. 3). Thylakoids are normally separated from one another by 35 to 45 nm. The space is occupied in part by regular arrays of disk-shaped phycobilisomes attached to the outer surfaces of each thylakoid.

The structure of the nucleoplasm is similar to that of other procaryotic organisms (Fig. 2). Most of the cytoplasmic inclusions that occur in other groups of cyanobacteria are evident in the cells of the *Pleurocapsales*. These include: carboxysomes (35–37) (Fig. 2), often associated with the nucleoplasm; glycogen granules (Fig. 1); and cyanophycin granules and polyphosphate granules (not illustrated).

Freshly released baeocytes cannot be distinguished from mature vegetative cells by their internal fine structure. However, the F layer of the cell wall is either absent or thinner than that of the vegetative cell (Fig. 8).

PATTERNS OF DEVELOPMENT

Developmental patterns in the *Pleurocapsales* are determined by three principal parameters: the relative importance of binary and multiple fission, the planes of successive binary fissions, and the suppression or maintenance of F-layer synthesis during multiple fission (leading, respectively, to motility or immotility of baeocytes). Among the strains that we have examined, six different developmental patterns can be distinguished. With an important exception to be discussed below, each developmental pattern is characteristic of a described pleurocapsalean genus. We have attached an existing generic name to five strain clusters distinguishable by developmental properties (Table 4). Strain assignments to *Dermocarpa, Xenococcus, Dermocarpella, Myxosarcina,* and *Chroococcidiopsis* are clear-cut and correspond well to present taxonomic practice. However, some of the differential characters listed in Table 4 are new, since they had not been recognized before the present study. They include the absence of binary fission in *Xenococcus*, the occurrence of binary fission in *Chroococcidiopsis*, and the presence or absence of baeocyte motility.

One strain cluster, the *Pleurocapsa* group, presents a special taxonomic problem. Cyanobacteria that display this general developmental pattern are currently placed in a variety of genera, which include *Pleurocapsa, Hyella, Radaisia, Onkonema,* and *Tryponema*. The distinctions between these genera are made on the basis either of characters not easily determinable with cultures (the endolithic growth habit) or of characters that are subject to considerable environmental variation in cultured material (the arrangement of vegetative cells in aggregates). As a provisional measure, we shall apply the designation "the *Pleurocapsa* group" to this large and internally diverse strain cluster.

The following accounts of developmental patterns in each group are based on semicontinuous observations of baeocyte development in Cooper dish cultures, and on the electron microscopic examination of thin sections of material fixed at various growth stages. Only on the basis of such observations is it possible to interpret adequately the various growth stages observable in

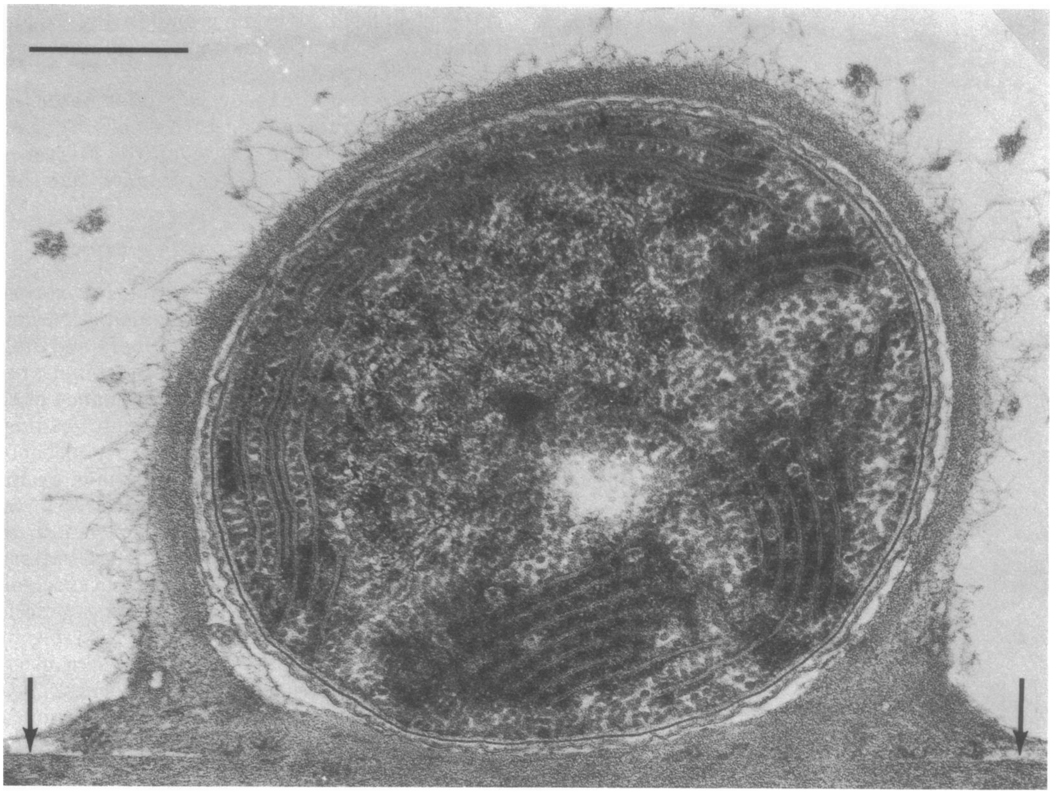

FIG. 7. *Electron micrograph of a thin section of Dermocarpella at an early stage of growth on a piece of dialysis membrane placed on the surface of a plate of medium MN. Arrows indicate the surface of the dialysis membrane. The F layer of the cell wall flares out to produce a broad basal attachment of the cell to the dialysis membrane.*

mass cultures. In groups such as *Myxosarcina*, *Chroococcidiopsis*, and members of the *Pleurocapsa* group, which produce large aggregates of vegetative cells with a distinctive arrangement, the characteristic and group-specific arrangement is almost always more evident in material grown on agar plates than in liquid cultures.

Dermocarpa

Four strains of marine origin (7301, 7302, 7303, and 7304) and two freshwater strains (7437 and 7438) are assignable to the genus *Dermocarpa*. Mass cultures are composed of spherical cells of varying size, some reaching 20 to 30 μm in diameter just before multiple fission. Most cells appear homogeneous by light microscopy; some of the larger ones are filled with baeocytes (Fig. 9).

The development of strain 7302 grown in a Cooper dish is shown in Fig. 10. Baeocytes enlarge symmetrically into spherical vegetative cells, which increase in size until the onset of multiple fission. The baeocytes are motile after release from the parental cell, but, since the culture shown in Fig. 10 was subjected to uniform illumination, their migration is random. The directed movement in a light gradient of a baeocyte population of *Dermocarpa* strain 7302 has been previously described (see Fig. 5).

Cleavage cannot normally be detected by light microscopy, and its successive stages have been studied by electron microscopic examination of thin sections (Fig. 11 and 12). Shortly before the onset of cleavage, vegetative cells are surrounded by a very thick F layer and contain numerous discrete nuclei; at this stage, the three wall layers are still closely appressed to one another (Fig. 11a). As cleavage begins, the PG and OM layers retract from the F layer, which does not participate in the cleavage process. Cleavage occurs through the formation of transverse walls composed of the PG and OM layers, and only these wall layers are present around the baeocytes at the termination of division. No new F-layer material is synthesized within the parental cell during cleavage.

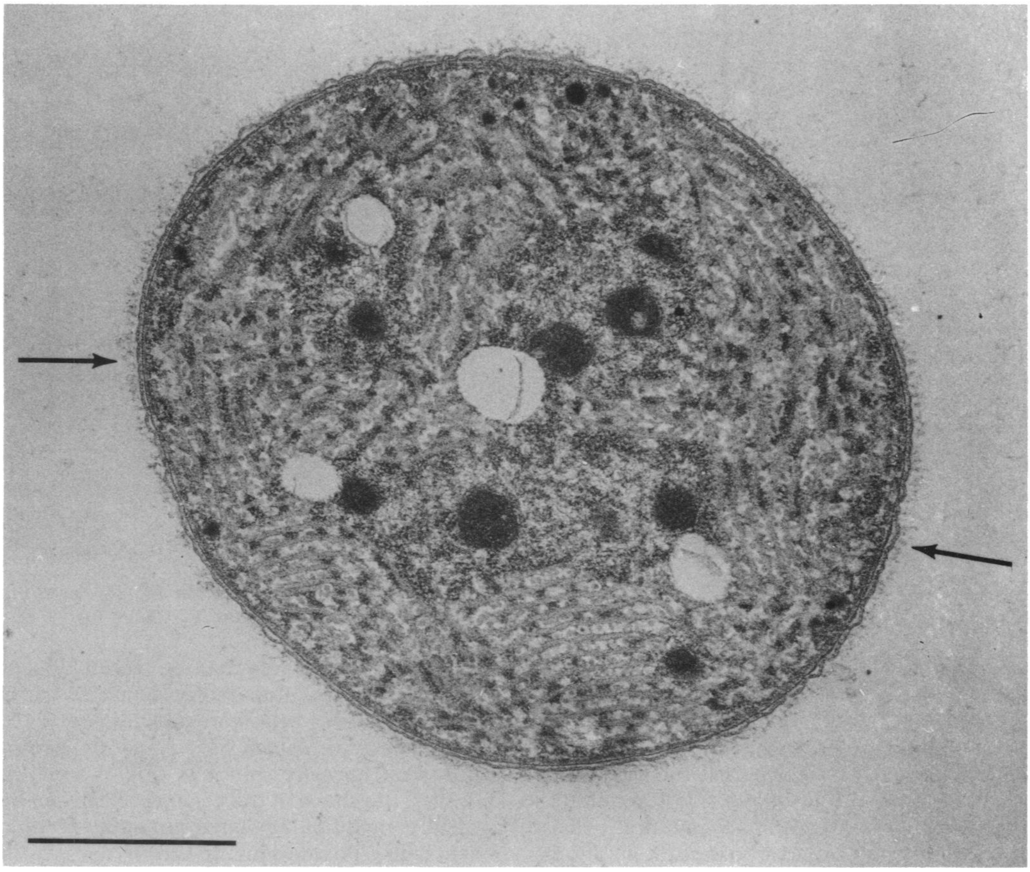

FIG. 8. *Electron micrograph of a thin section of a freshly released baeocyte of a member of the Pleurocapsa group (7310). Synthesis of the F layer (arrows) has just commenced.*

TABLE 4. *Key to the subgroups of the Pleurocapsales*

I. *Cell division solely by multiple fission*
  A. Baeocytes motile .... *Dermocarpa*
  B. Baeocytes immotile .... *Xenococcus*
II. *Cell division by a combination of binary and multiple fission*
  A. Baeocyte development leads to formation of a vegetative cell, which undergoes one to three binary fissions, to produce a single apical cell, which divides by multiple fission and releases baeocytes; the basal cell(s) subsequently enlarges and repeats the cycle .... *Dermocarpella*
  B. Baeocyte development followed by repeated binary fissions in three planes at right angles to one another, producing a cubical aggregate of cells, all of which normally undergo multiple fission.
    1. Baeocytes motile .... *Myxosarcina*
    2. Baeocytes immotile .... *Chroococcidiopsis*
  C. Baeocyte development followed by repeated binary fissions in many different planes, to produce irregular, sometimes filamentous aggregates of cells. Some or all of the cells in the aggregate undergo multiple fission .... *Pleurocapsa group*

The size of the vegetative cell at the onset of cleavage varies widely within each strain and is, clearly, much influenced by environmental factors. For example, if a culture in the stationary phase is transferred to fresh medium, a large fraction of the vegetative cells in the population, irrespective of size, will cleave and release baeocytes within 12 to 24 h. Freshly released baeocytes are practically uniform in size in each strain but may vary in size between strains. Our strains fall into two clusters with respect to baeocyte size: those having baeocytes that range from 1.5 to 2.0 μm (7302, 7303, and 7304) and those having baeocytes that range from 3.0 to 4.0 μm (7301, 7437, and 7438).

Multiple fission in *Dermocarpa* normally results in a total conversion of the contents of the parental cell into baeocytes; no cytoplasmic material remains within the F layer after baeocytes have been released. Rarely, accidents of division occur; such cells contain a large, undivided segment of cytoplasm, together with many baeocytes. They can occasionally be detected by light

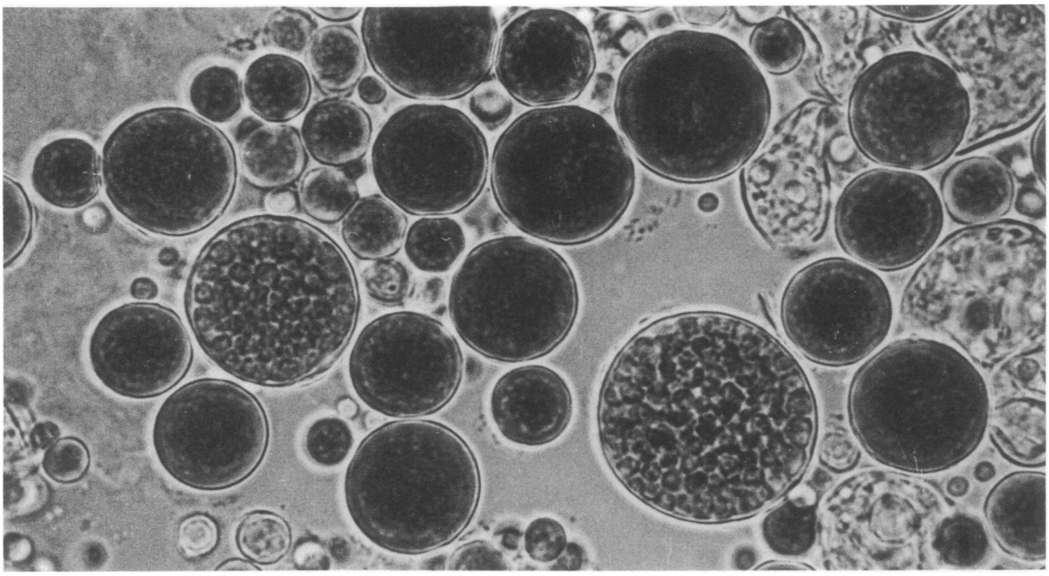

FIG. 9. *Light micrograph of a mass culture of Dermocarpa (7437), grown on medium BG-11, revealing spherical cells of varying size that appear either homogeneous or filled with baeocytes. ×1,000.*

microscopy in mass cultures, and one has been observed by electron microscopy in a thin section (Fig. 11b).

Since the size of the vegetative cell at the onset of cleavage varies, whereas the size of the baeocyte is constant, it follows that the number of baeocytes produced from a parental cell is variable. This number can be accurately determined by examining single bursts in Cooper dish cultures; a series of values for each of five strains is shown in Table 5. As expected, the intrastrain variation is very large.

If baeocytes were formed through a series of synchronous cell divisions, the recorded burst sizes should all fall in the series 2^n, where n represents the number of successive divisions undergone by the parental cell. In fact, very few of them do, and many of the burst sizes recorded in Table 5 are roughly intermediate (e.g., 21, 23, 95, 181, 206, and 340) between two successive numbers of the series.

The numerous nuclei present in vegetative cells that have not yet started to divide (Fig. 11a) suggests that nuclear division may terminate before the onset of multiple fission. In this event, the number of viable baeocytes produced can be no greater than the number of nuclei present at the start of division, and multiple fission might give rise to some anucleate cells. On one occasion, observation of the baeocytes released from a single parental cell of strain 7301 was continued until vegetative development was well underway. Of the 281 baeocytes released, only 256 (2^8) subsequently gave rise to vegetative cells; a surprisingly large fraction (about 10%) of the population was, apparently, nonviable. It is possible that these cells were anucleate.

The vegetative cell in all strains of *Dermocarpa* is intrinsically spherical (Fig. 9 and 10). However, the shape of the vegetative cell can be markedly modified by environmental factors. When dense populations of spores develop in attachment to a common substrate (for example, the glass wall of a culture vessel), the vegetative cells are subjected to mutual compression and assume pyriform or clavate shapes (Fig. 13). Cells of this form are frequent in natural *Dermocarpa* populations and have led many observers to the incorrect conclusion that the vegetative cell of *Dermocarpa* possesses an intrinsic polarity.

Xenococcus

Three strains, all of marine origin (7305, 7306, and 7307), are assignable to the genus *Xenococcus*. Mass cultures examined by light microscopy are composed of a mixture of uncleaved cells of widely varying size, large cells in the course of division, and large cells filled with baeocytes (Fig. 14). Baeocytes of individual strains are of uniform size, typically measuring 2 to 3 μm in diameter, whereas the diameter of vegetative cells is variable and may reach 25 μm prior to multiple fission.

The development of strain 7305 in a Cooper dish is shown in Fig. 15. Following symmetrical baeocyte enlargement, the spherical vegetative cell underwent cleavage (the stages of which

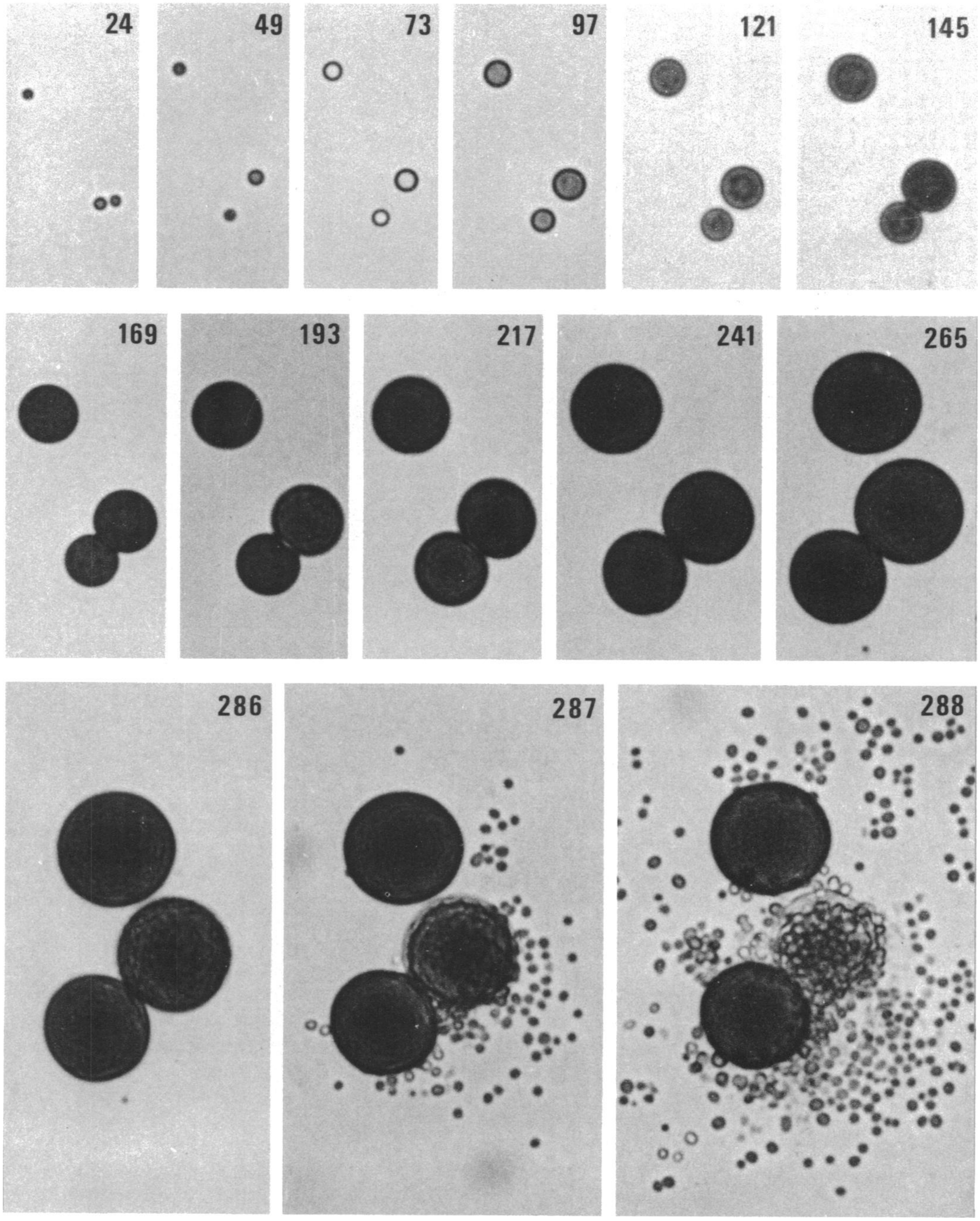

FIG. 10. *Development of Dermocarpa (7302) on medium MN in a Cooper dish culture. ×500. (In plates illustrating the development of Cooper dish cultures, the number on each photomicrograph indicates the elapsed time, in hours, after the initial observation.)*

cannot be resolved by this method of examination) and released 14 baeocytes. Since the baeocytes of *Xenococcus* are immotile, they remain clustered around the parental F layer after release (Fig. 6a and b) and enlarge into vegetative cells. At the end of the observation period, baeocytes of the second generation had begun to be released.

Figures 16 and 17 are electron micrographs of thin sections of a series of cleavage stages in

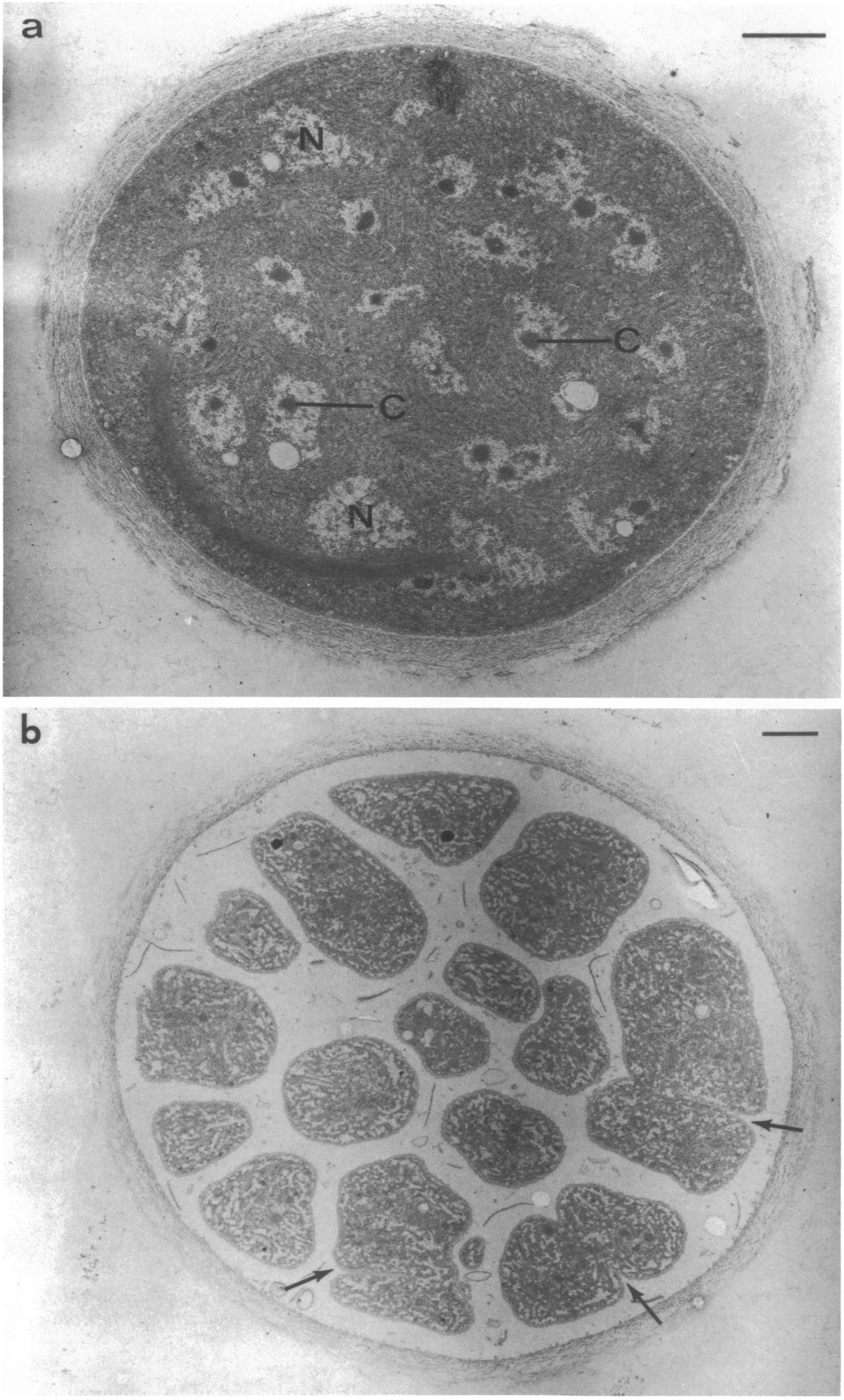

FIG. 11. *Electron micrographs of thin sections of Dermocarpa (7304) grown in liquid medium MN. (a) Cell just before the onset of multiple fission; it contains many separate nucleoids (N), frequently associated with carboxysomes (C). (b) Cell in the course of multiple fission; the cleavage products have retracted from the parental F layer. Dividing cells are indicated by arrows.*

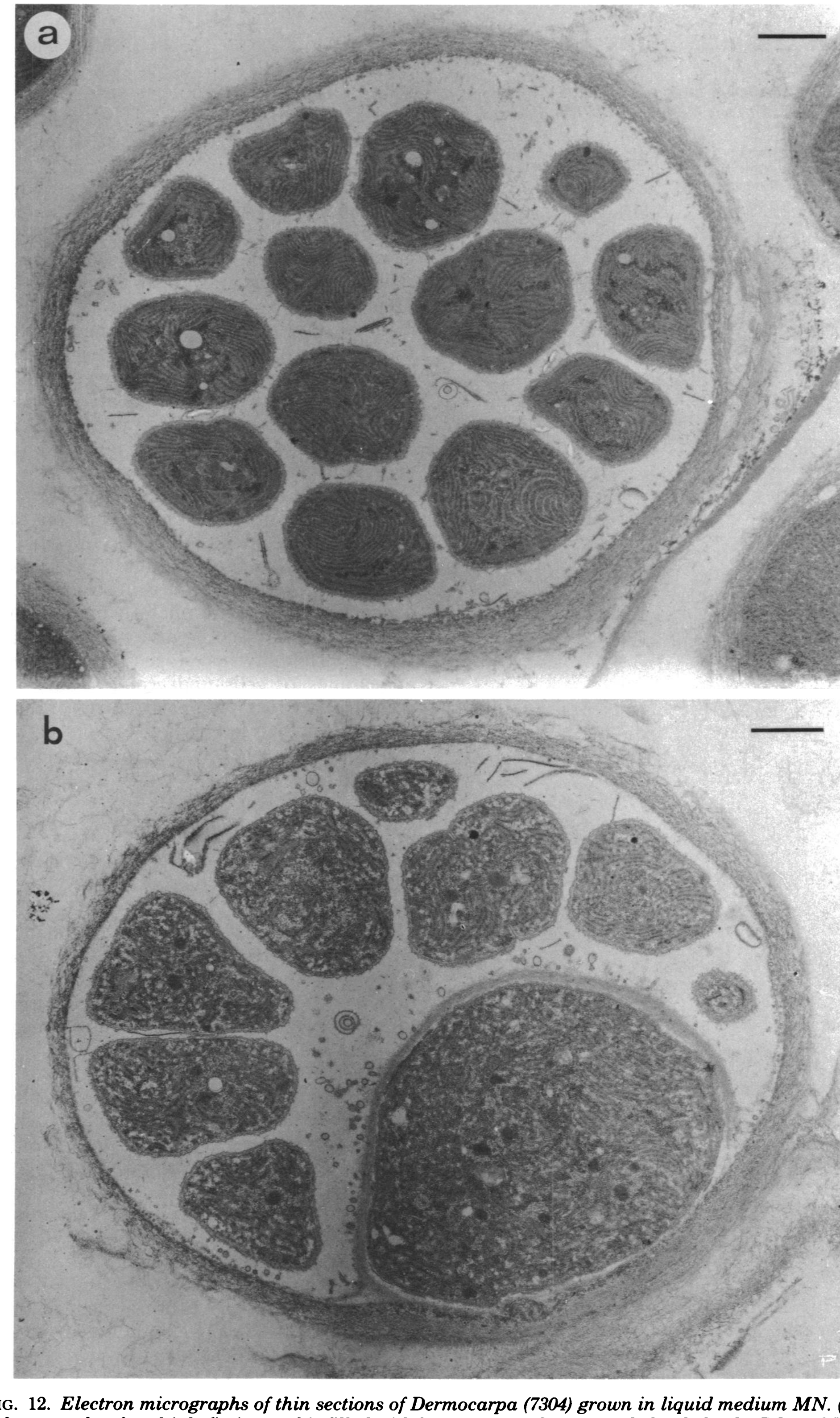

FIG. 12. *Electron micrographs of thin sections of Dermocarpa (7304) grown in liquid medium MN. (a) Cell that has completed multiple fission and is filled with baeocytes, each surrounded only by the PG and LP wall layers. (b) Cell undergoing multiple fission that contains a large, uncleaved segment of cytoplasm surrounded by an F layer.*

TABLE 5. *Number of baeocytes released by single parental cells of Dermocarpa and Dermocarpella strains*

Strain	Total no. of determinations	No. of baeocytes released	
		Range	Individual values
Dermocarpa			
7301	11	16–293	16, 16, 21, 21, 23, 23, 30, 32, 32, 281, 293
7302	9	70–1,048	70, 71, 78, 80, 95, 155, 206, 340, 1,048
7303	9	181–868	181, 458, 540, 594, 595, 716, 845, 853, 868
7304	6	67–199	67, 163, 171, 174, 180, 199
7437	15	116–267	116, 120, 130, 137, 142, 152, 158, 160, 163, 192, 213, 243, 247, 263, 267
Dermocarpella			
7326	7	60–120	60, 76, 80, 94, 101, 107, 120

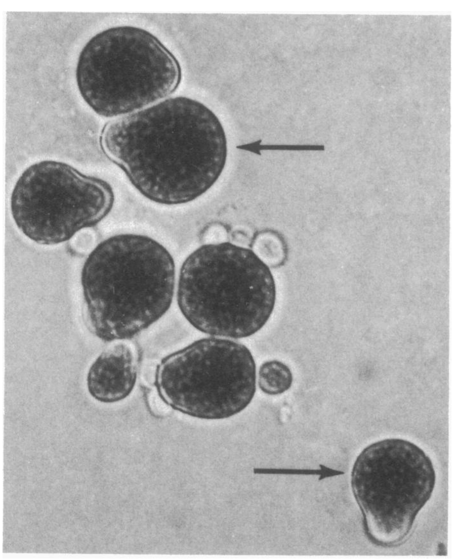

FIG. 13. *Light micrograph of Dermocarpa (7302), showing pyriform cells (arrows) produced through mutual compression when developing under crowded conditions attached to a substrate. Preparation from a culture grown in an unagitated flask of medium MN, where many cells grow in dense masses attached to the wall of the flask.* ×*1,000.*

strain 3707. Before the onset of multiple fission, the three wall layers are closely appressed, and the F layer is as much as 400 nm thick (Fig. 16a). As cleavage begins, the PG and OM layers retract from the F layer (Fig. 16b). The cleavage products, formed by ingrowth of the PG and OM layers, start to synthesize new F-layer material. When cleavage is complete, each baeocyte is enclosed by an individual F layer, and this material fills much of the interstitial space (Fig. 17b).

Just as in *Dermocarpa*, the size of freshly released baeocytes is constant, whereas the size of vegetative cells at the onset of multiple fission is variable within each strain. Burst sizes consequently vary a great deal, ranging in strain 7305 from 4 to over 100 baeocytes. In Cooper dish cultures, the burst size at the first generation is usually between 4 and 16, but it is considerably greater at the second generation, when development occurs under relatively crowded conditions as a result of the immotility of the baeocytes. This suggests that favorable growth conditions lead to early cleavage.

In summary, the development of *Xenococcus* parallels that of *Dermocarpa* with one difference: synthesis of the F layer occurs during the course of baeocyte formation in *Xenococcus*, whereas in *Dermocarpa* it is delayed until after baeocyte release. The microscopic appearance of a mass culture is deceptive, since some of the readily visible division stages (Fig. 14) give the appearance of cells undergoing binary fission when, in fact, they are early stages of multiple fission. This had led earlier workers (3, 14, 34) to conclude, incorrectly, that *Xenococcus* can undergo binary fission.

Dermocarpella

The genus *Dermocarpella* is represented in our collection by one marine strain, 7326. Light microscopic examination of a mass culture reveals a population composed of small cells, which are spherical or nearly so, and of larger, ovoid cells (Fig. 18). Some of the latter have undergone division and contain one large and one small cell; others contain a mass of baeocytes together with one or two larger cells.

Figure 19 shows the development of this strain in a Cooper dish culture. The baeocyte, spherical and approximately 2 μm in diameter, enlarges into an ovoid vegetative cell, which gradually becomes pyriform, measuring about 10 by 13 μm just before baeocyte release. The details of cell division cannot be clearly observed; however, the structure that remains after baeocyte release still contains cellular material. The baeocytes are motile and respond phototactically to a light gradient. This feature is not evident in Fig. 19, since the culture was evenly illuminated.

The course of cell division has been elucidated by electron microscopy of thin sections (Fig. 20 and 21). Before the onset of division, the cell is enclosed by an F layer about 300 nm thick, closely appressed to the OM layer. The first

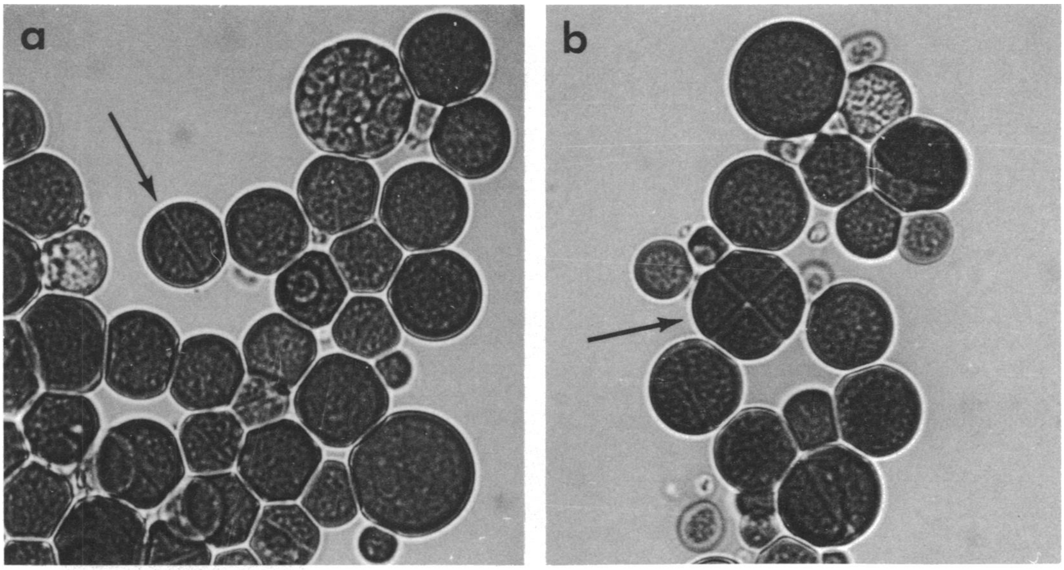

FIG. 14. *Light micrographs of a mass culture of Xenococcus (7306) grown on agar plates of medium MN. Arrows point to dividing cells. ×1,000.*

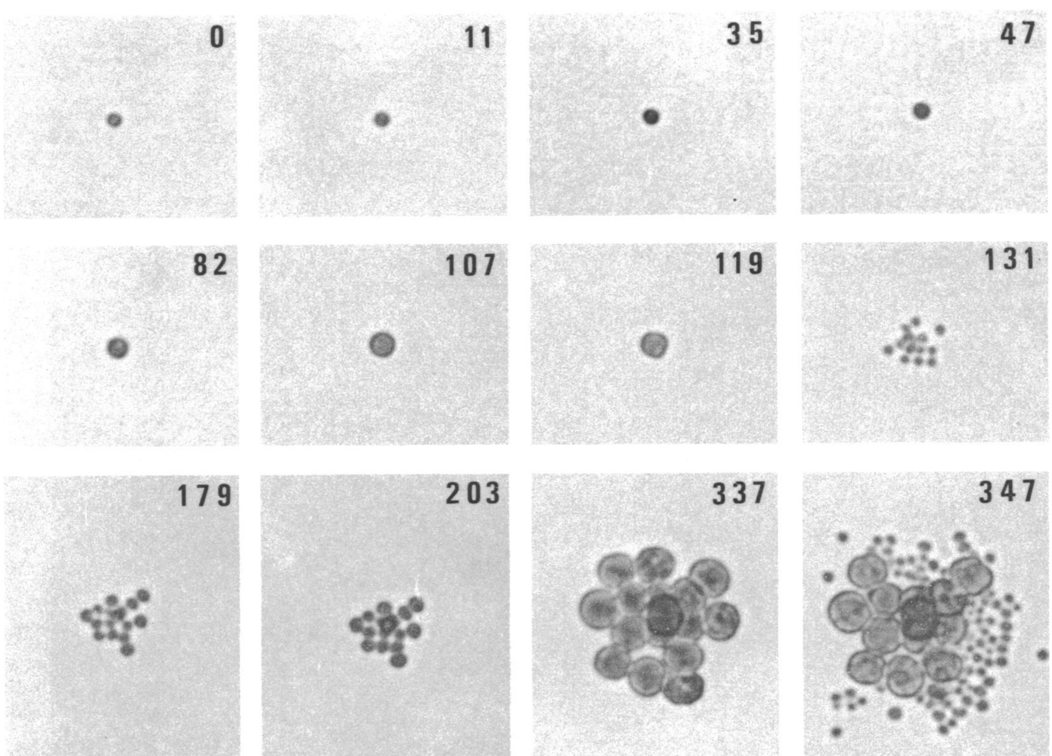

FIG. 15. *Development of Xenococcus (7305) on medium MN in a Cooper dish culture. The successive divisions that lead to baeocyte formation (see Fig. 14) are not visible in this preparation as a result of the relatively poor resolution. ×500.*

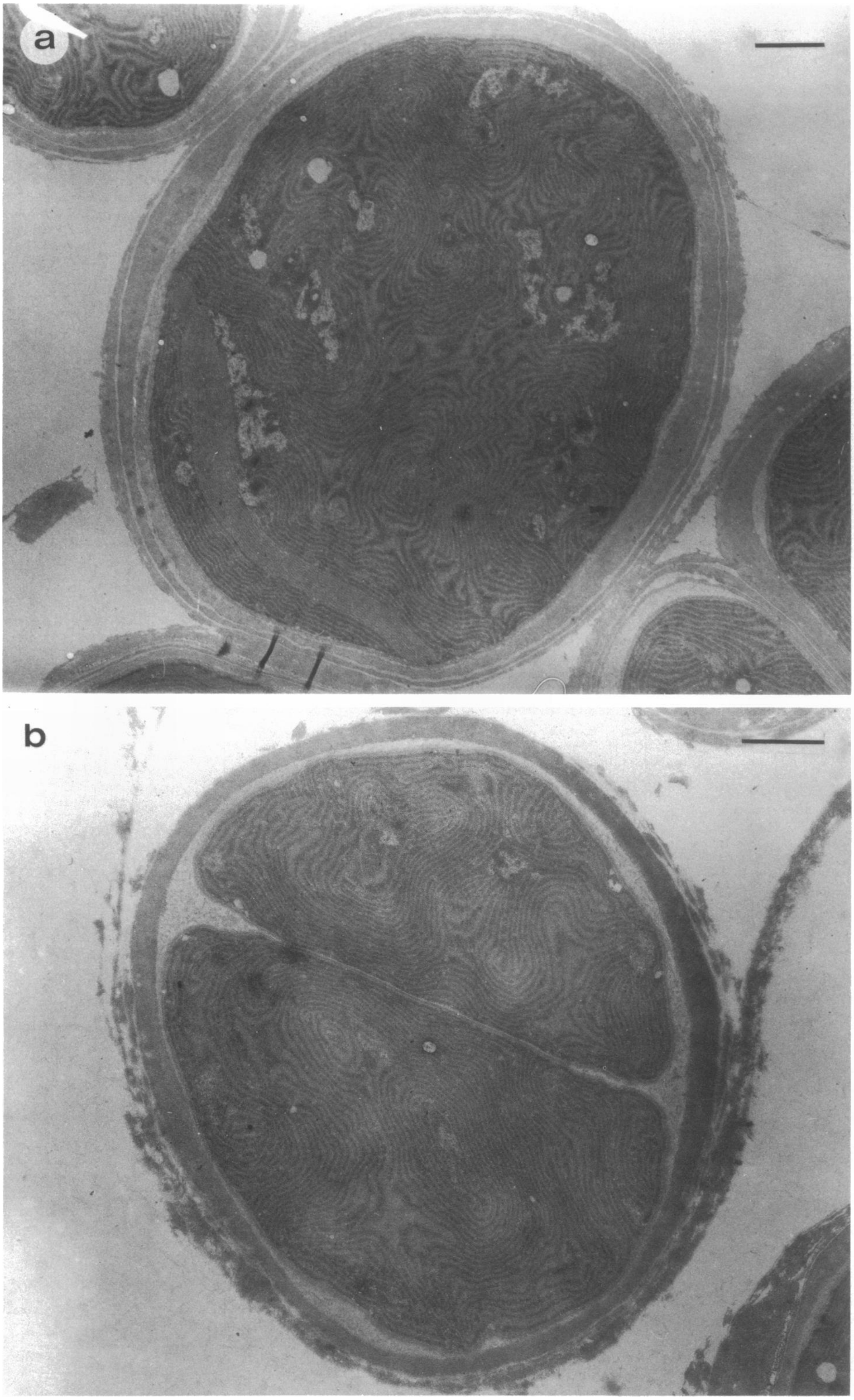

FIG. 16. *Electron micrographs of thin sections of Xenococcus (7307) grown in an agar overlay on medium MN. (a) Cell before the onset of multiple fission; the PG, OM, and F layers are closely appressed to one another. (b) Two-cell stage of multiple fission; note that the daughter cells have retracted from the F layer of the parental cell wall.*

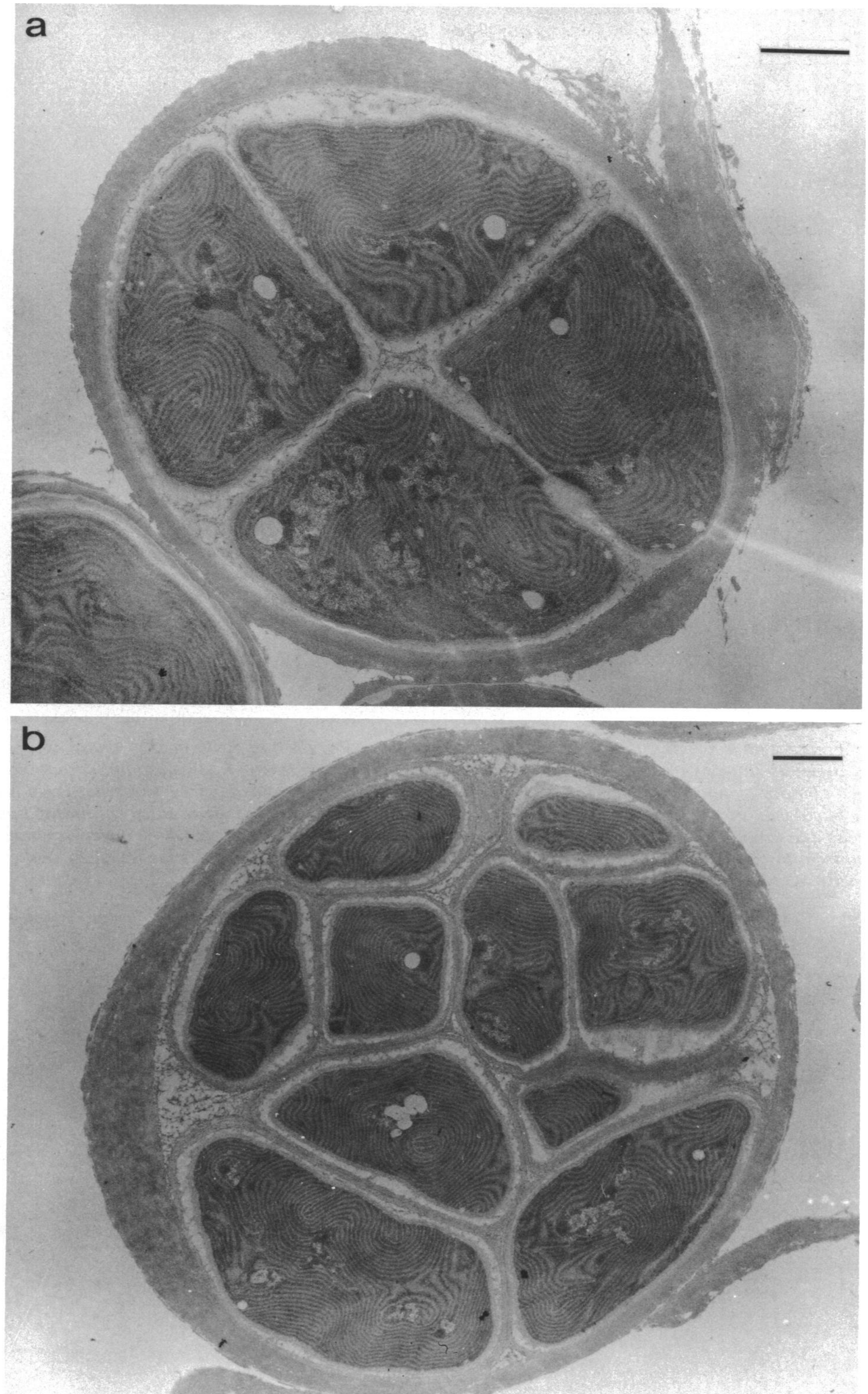

FIG. 17. *Electron micrographs of thin sections of Xenococcus (7307) from the same preparation as the cells in Fig. 16. (a) Four-cell stage of multiple fission. (b) Cell that has cleaved into numerous baeocytes, each surrounded by a distinct F layer.*

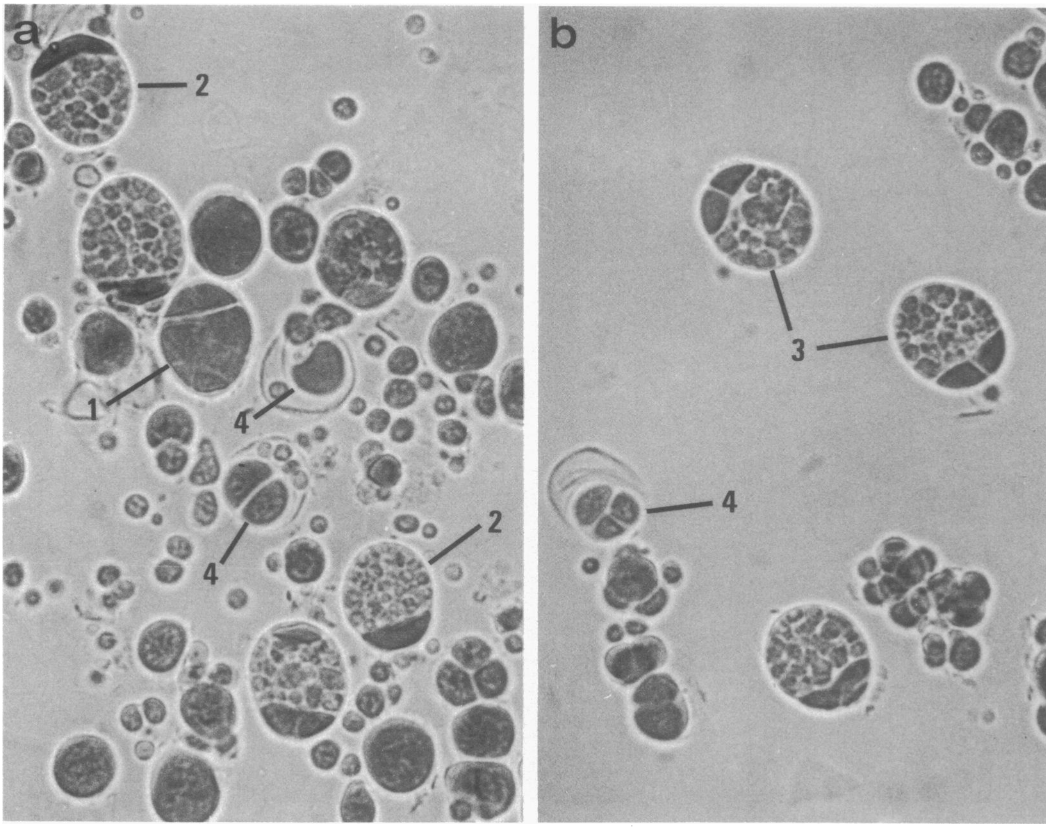

FIG. 18. *Light micrographs of a liquid culture of Dermocarpella (7326) in medium MN. These figures show various division stages that cannot be resolved in Cooper dish cultures: 1, an individual that has just undergone binary transverse fission to form a small basal and a large apical cell; 2, individuals containing a single basal cell and an apical cell that has completed multiple fission; 3, individuals containing a pair of basal cells and an apical cell that has completed multiple fission; 4, individuals in which baeocytes have been released from the apical cell, revealing the outline of the parental F wall layer, which also encloses the basal cell or cells. ×1,000.*

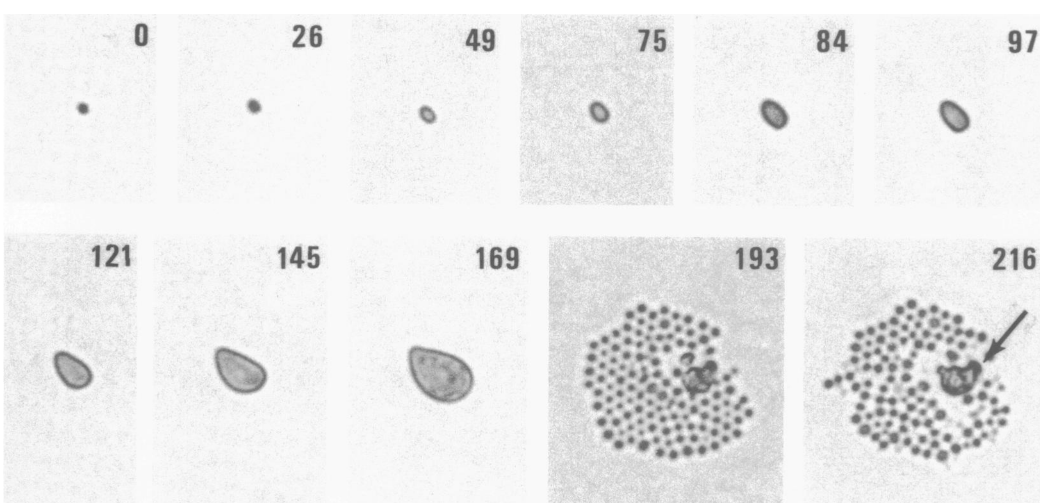

FIG. 19. *Development of Dermocarpella (7326) on medium MN in a Cooper dish culture. Asymmetric enlargement of the baeocyte leads, after 169 h, to the formation of a large ovoid cell, which has probably already undergone binary fission, although the transverse wall is not resolved. Arrow points to the basal cell remaining after baeocyte release. ×500.*

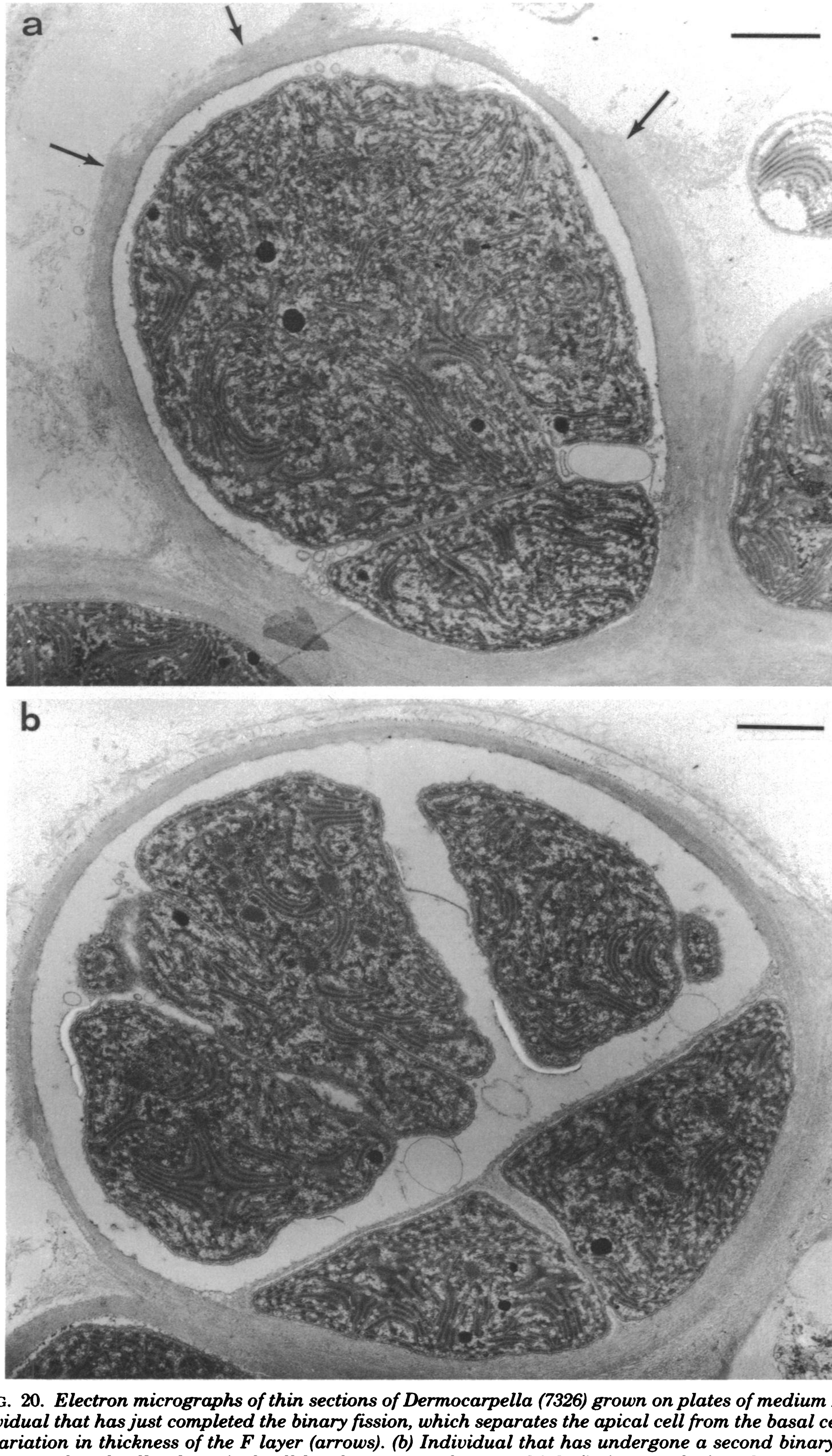

FIG. 20. *Electron micrographs of thin sections of Dermocarpella (7326) grown on plates of medium MN. (a) Individual that has just completed the binary fission, which separates the apical cell from the basal cell; note the variation in thickness of the F layer (arrows). (b) Individual that has undergone a second binary fission to form two basal cells; the apical cell has begun to undergo multiple fission, producing cleavage products that are, initially, not of equal size; the basal cells have begun to synthesize new F-layer material over their entire surfaces, but no new synthesis of F-layer material has occurred around the dividing cells of the apical portion.*

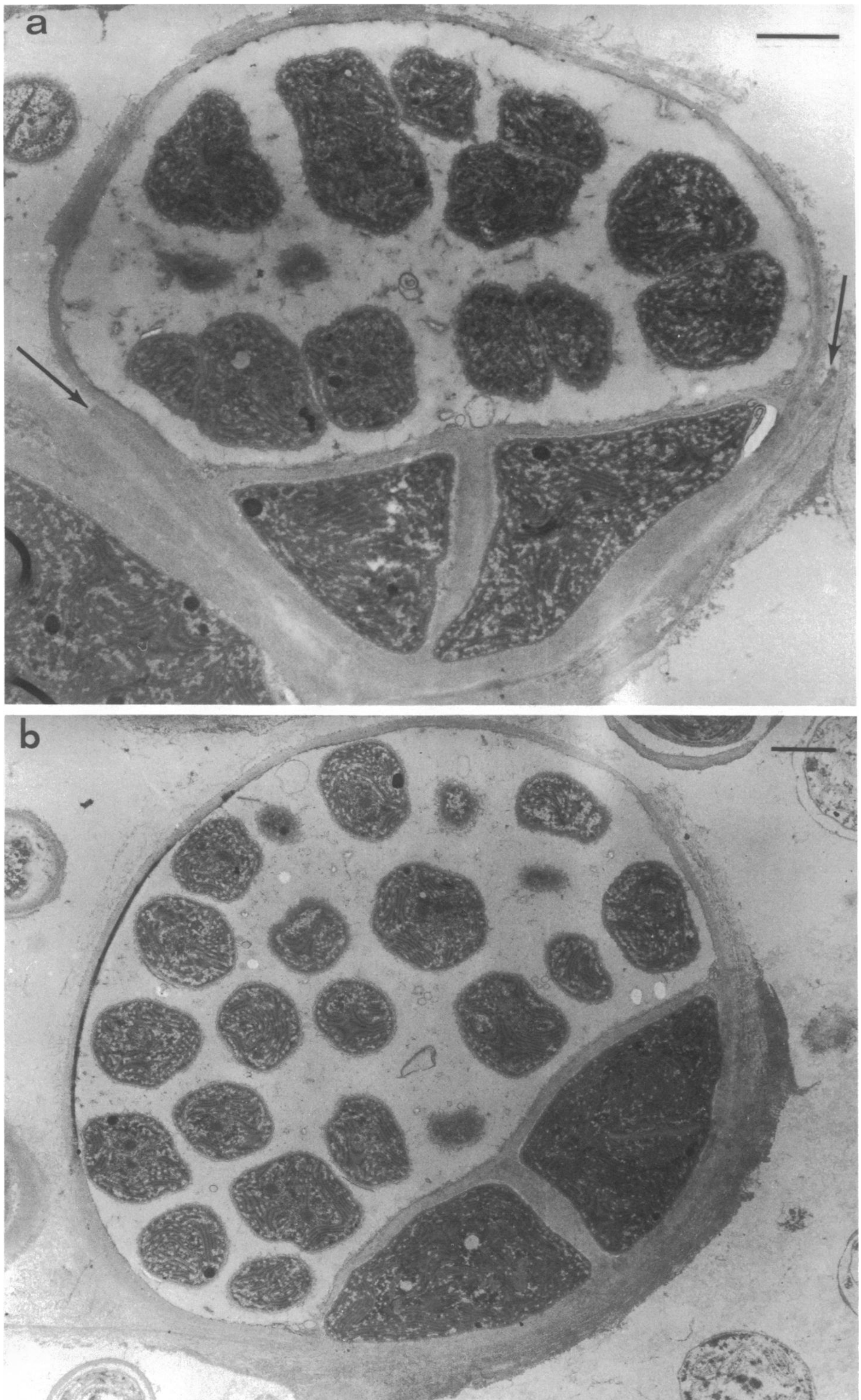

FIG. 21. *Electron micrographs of thin sections of Dermocarpella (7326) from the same preparation as the cells illustrated in Fig. 26. (a) Individual nearing completion of multiple fission; the F layer surrounding the basal cells is markedly thicker than that in the earlier developmental stage shown in Fig. 20b; no F-layer material surrounds the cleavage products in the apical cell (note the variation in thickness of the enclosing F layer [arrows]). (b) Individual that has completed multiple fission and is filled with baeocytes.*

plane of cleavage is transverse and produces a small vegetative cell and a much larger reproductive cell. Both during and after this division, the wall of the daughter vegetative cell remains closely appressed to the F layer of the parental cell; the wall of the reproductive cell, on the other hand, begins to retract from the F layer of the parental cell even before separation from the daughter vegetative cell is complete. The daughter vegetative cell immediately begins to resynthesize F-layer material over its entire surface. It may undergo a second or third division, at right angles to one another (Fig. 18). If this occurs, new F-layer material is also intercalated between the cells so produced (Fig. 20b and 21). The reproductive cell begins to undergo rapid successive cleavages, producing a large population of baeocytes. During multiple fission, synthesis of the F layer in the reproductive cell is suppressed, with the result that the baeocytes are individually enclosed only by the PG and OM wall layers at the time of release.

After rupture of the F layer of the reproductive cell and release of the enclosed baeocytes, the vegetative cell (or cells) remains at the base of the now-empty F layer, to which it is firmly attached, and begins to enlarge. This leads to a repetition of the distinctive division cycle described above. However, since the developing vegetative cell is enclosed by a newly synthesized F layer and also attached basally to the F layer formed at the preceding generation, the thickness of the F-layer material is considerably greater at its base than at its apex. This feature of wall structure, distinctive of *Dermocarpella*, is evident in Fig. 20b and 21.

Dermocarpella accordingly displays an intrinsic polarity, expressed by the asymmetric enlargement of the vegetative cell, by its subsequent asymmetric division, and by the different fates of the two daughter cells so produced. In this respect, it differs fundamentally from *Dermocarpa* and, indeed, from all other pleurocapsalean cyanobacteria.

The polarity of development could, in principle, be determined by the site at which the baeocyte attaches to the substrate. We have, accordingly, examined the development of vegetative cells produced from baeocytes attached to a sheet of dialyzing membrane deposited on the surface of an agar plate. Electron micrographs of thin sections show that the planes of elongation and division are completely unrelated to the plane of the underlying substrate. An example of a cell attached apically to the substrate is shown in Fig. 22. Thus, the polarity of growth and cell division is not determined by the site of baeocyte attachment.

The variation of burst size in *Dermocarpella* is much less than that in *Dermocarpa* and *Xenococcus* (Table 5). This reflects the relative constancy of size of the vegetative cell at the time when multiple fission begins.

Myxosarcina

Myxosarcina is represented in our collection by two strains, 7312 and 7325, both of marine origin.

The developmental cycle observed in Cooper dish cultures, similar in the two strains, is illustrated for strain 7312 in Fig. 23. The baeocyte (2 to 3 μm in diameter) enlarges into a vegetative cell of fixed strain-specific size (5 μm for strain 7312 and 8 to 10 μm for strain 7325) that then begins to divide by binary fission. Many successive divisions take place in three alternating planes, to produce a large and fairly regular cubical aggregate. When it occurs, multiple fission is massive; almost all the cells of the aggregate cleave and release baeocytes, which are motile after release.

Since the sizes of the vegetative cell and the baeocyte are virtually constant in each strain, the burst size (number of baeocytes) is probably also constant. It is relatively small, and, although difficult tc determine with precision, the value is approximately four in strain 7312 and somewhat greater in strain 7325.

Chroococcidiopsis

All *Chroococcidiopsis* strains in our collection are of freshwater or terrestrial origin. Seven (7203, 7431, 7432, 7433, 7434, 7436, and 7439) are structurally indistinguishable, having baeocytes 3 μm in diameter and vegetative cells 5 μm in diameter just before the onset of vegetative division. Strain 6712 differs from these strains in the sizes of baeocytes (4 μm) and vegetative cells (6.3 μm).

Chroococcidiopsis is not distinguishable from *Myxosarcina* in mass cultures, where it develops as cubical or somewhat irregular cell aggregates (cf. Fig. 24 and 25b). When crushed, these aggregates break up into smaller cell clusters, most of which contain from two to eight vegetative cells in the course of binary fission (Fig. 25a). A few groups of baeocytes, recognizable by their smaller size, are indicated by arrows in Fig. 25a.

The developmental cycle characteristic of *Chroococcidiopsis* is difficult, if not impossible, to determine by examination of mass cultures but is revealed by semicontinuous observations of growth in Cooper dish cultures.

Figure 26 shows the development over a period of 229 h of a group of three baeocytes of strain 7432, representative of seven of the *Chroococcidiopsis* strains. The three baeocytes all en-

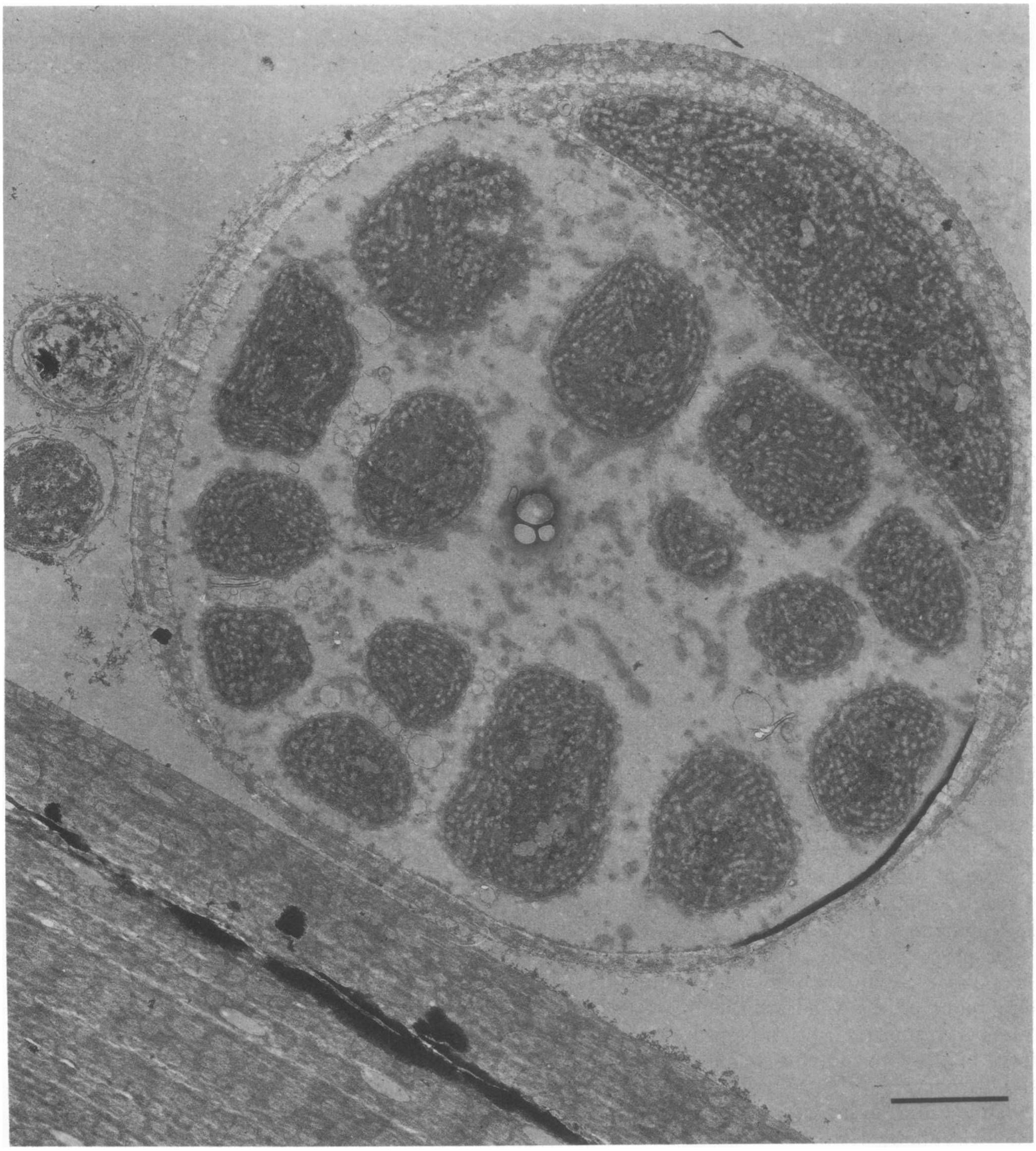

FIG. 22. *Electron micrograph of a thin section of Dermocarpella (7326) grown on a piece of dialysis membrane placed on a a plate of medium MN, showing an apically attached individual undergoing multiple fission.*

larged at similar rates and underwent the first binary fission after attaining the same size. Baeocytes were released from the aggregate after 157 h. After 208 h, many had enlarged and undergone one binary fission (arrows). After 229 h, some had divided a second time, to produce tetrads (arrows).

Baeocyte motility has never been observed in *Chroococcidiopsis*; it is this character that distinguishes *Chroococcidiopsis* from *Myxosarcina*. The immotility of the baeocytes of *Chroococcidiopsis* implies that synthesis of an F layer occurs in the course of multiple fission leading to baeocyte formation. The baeocytes of these organisms do not differ greatly in size from the vegetative cells, which indicates that a rather small (and probably constant) number of baeocytes is produced from each vegetative cell. Hence, the distinction between baeocytes and vegetative cells, either by light microscopy or by electron microscopy, is particularly difficult to make in this group and rests entirely on the

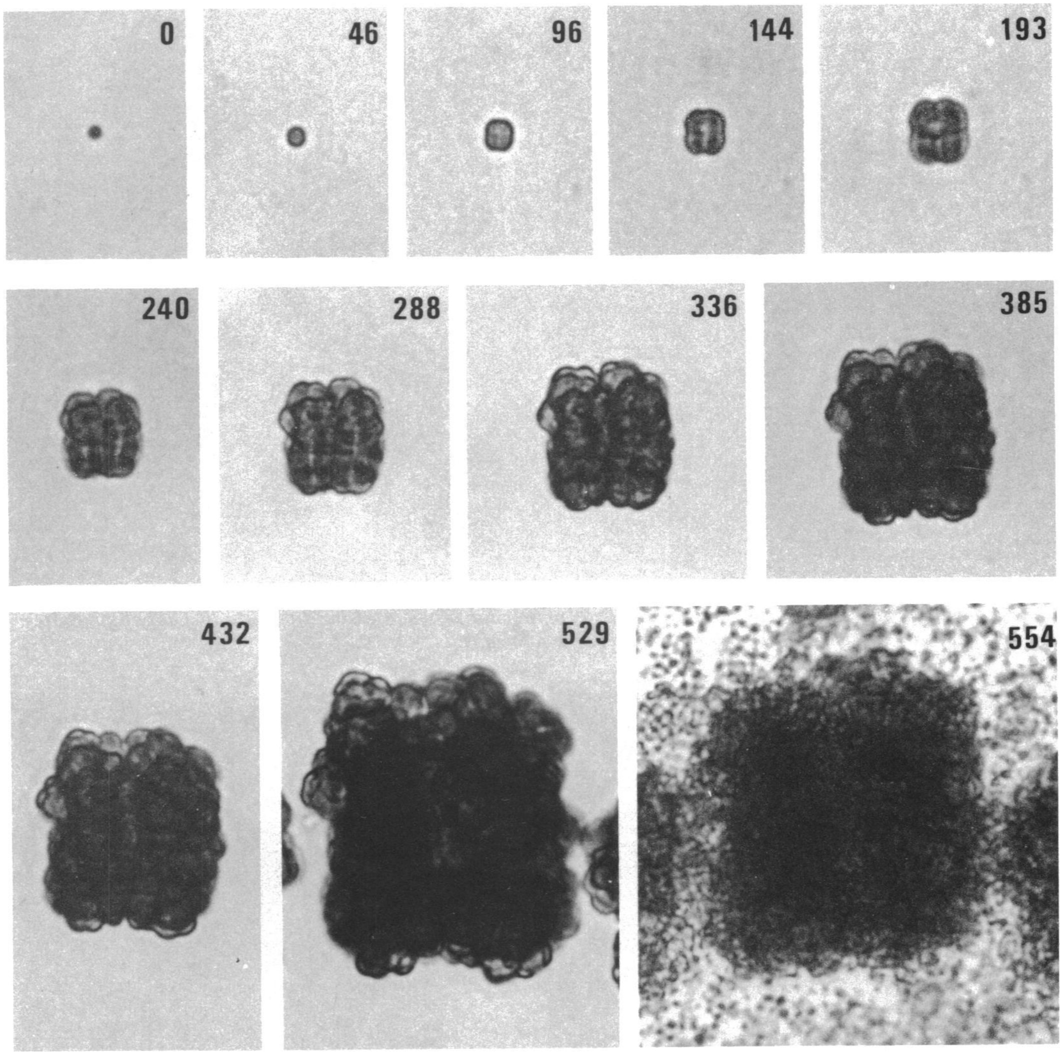

FIG. 23. *Development of a single baeocyte of Myxosarcina (7312) on medium MN in a Cooper dish culture. ×500.*

relatively small difference in cell size.

Electron micrographs of thin sections of *Chroococcidiopsis* aggregates are shown in Fig. 27. Figure 27a is a section of an aggregate that we interpret as having just undergone a binary fission, producing a two-celled individual. The individual cells are of approximately the same dimensions as cells in a Cooper dish culture that have just undergone the first division (Fig. 26, 61 h). The cell aggregate shown in Fig. 27b is of approximately the same size as that shown in Fig. 27a but contains many more cells. It can be interpreted as a two-celled aggregate, both cells of which are in the course of multiple fission. It should be noted that the cleavage products are individually surrounded by an F wall layer, in accord with the observation that free baeocytes are never motile.

Our interpretation of the developmental cycle of *Chroococcidiopsis* differs considerably from those offered by previous workers (12, 21, 22, 23), two of whom (Komárek and Hindák [23]) based their conclusions on the study of nonaxenic mass cultures of seven of the eight strains that we have examined. Earlier workers have assumed that *Chroococcidiopsis* is a unicellular organism that divides only by multiple fission, and they were thus forced to develop relatively complex explanations for the various types of cells and aggregates observable in mass cultures (12, 21, 23). The previous failure to recognize that *Chroococcidiopsis* can undergo both binary

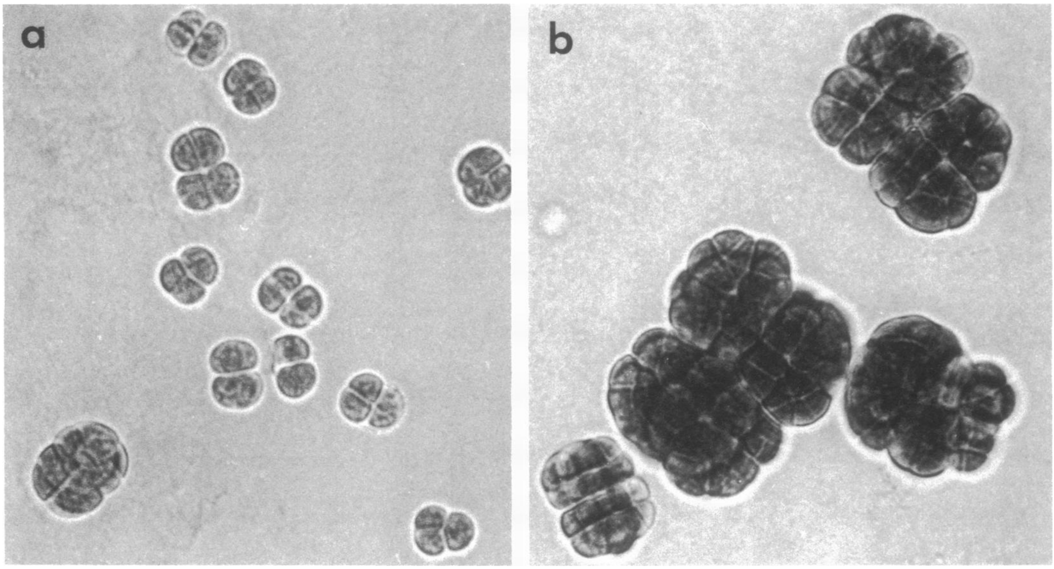

FIG. 24. *Light micrographs of Myxosarcina (7312) growing on a plate of medium MN. (a) Young aggregates, showing the uniformity of cell size and the regularity of the planes of successive divisions. (b) Older aggregates, showing the maintenance of fairly regular cubical packets of cells. ×1,000.*

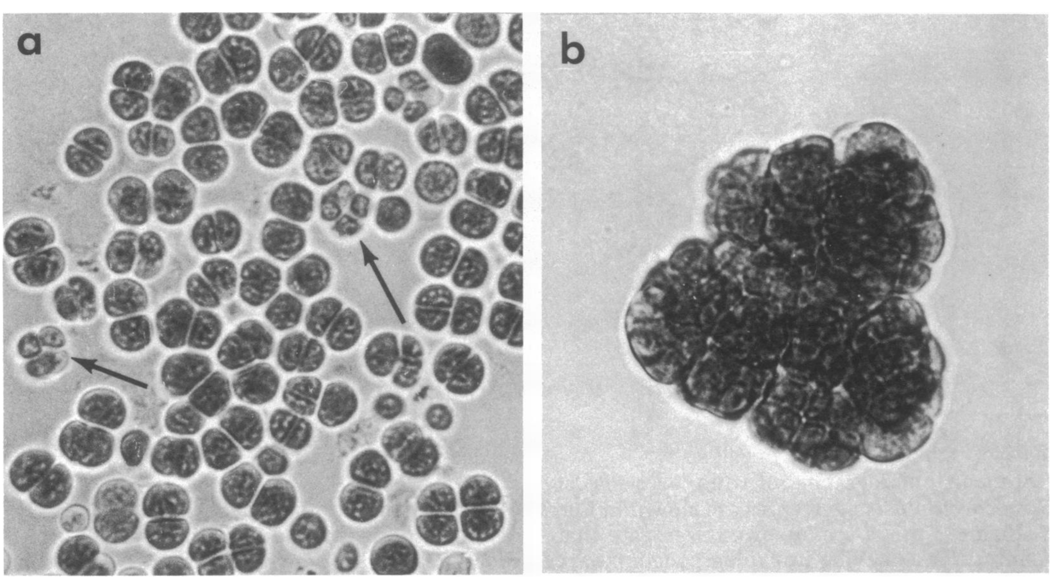

FIG. 25. *Light micrographs of mass cultures of Chroococcidiopsis grown on agar plates of medium BG-11. (a) Dispersed cells from a crushed cell aggregate of strain 6712, showing the regularity of cell size. Some of the cells (arrows) have undergone multiple fission, producing four baeocytes per parental cell. (b) Characteristic cell aggregate of strain 7431. ×1,000.*

fission and multiple fission accounts for the errors that have been made in the interpretation of its development.

Pleurocapsa Group

This *Pleurocapsa* group includes 11 strains of marine origin (7310, 7314, 7317, 7319, 7320, 7321, 7322, 7324, 7440, 7506, and 7516) and a moderate thermophile isolated from a hot spring (7327). Their development involves baeocyte enlargment followed by binary fission in many irregular planes, to produce cell aggregates that differ widely in size and complexity in different strains. Eventually, some cells in the aggregate undergo

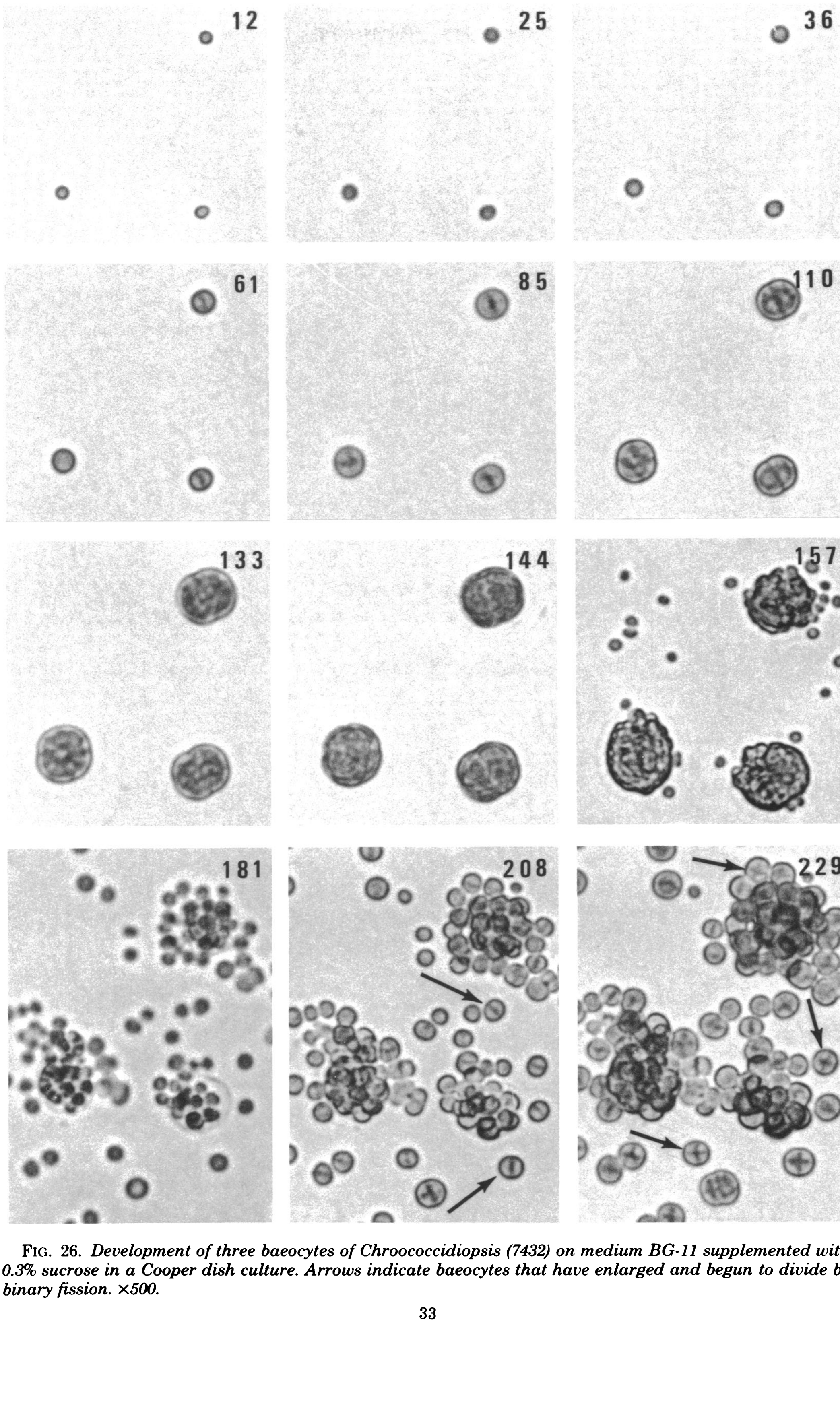

FIG. 26. *Development of three baeocytes of Chroococcidiopsis (7432) on medium BG-11 supplemented with 0.3% sucrose in a Cooper dish culture. Arrows indicate baeocytes that have enlarged and begun to divide by binary fission. ×500.*

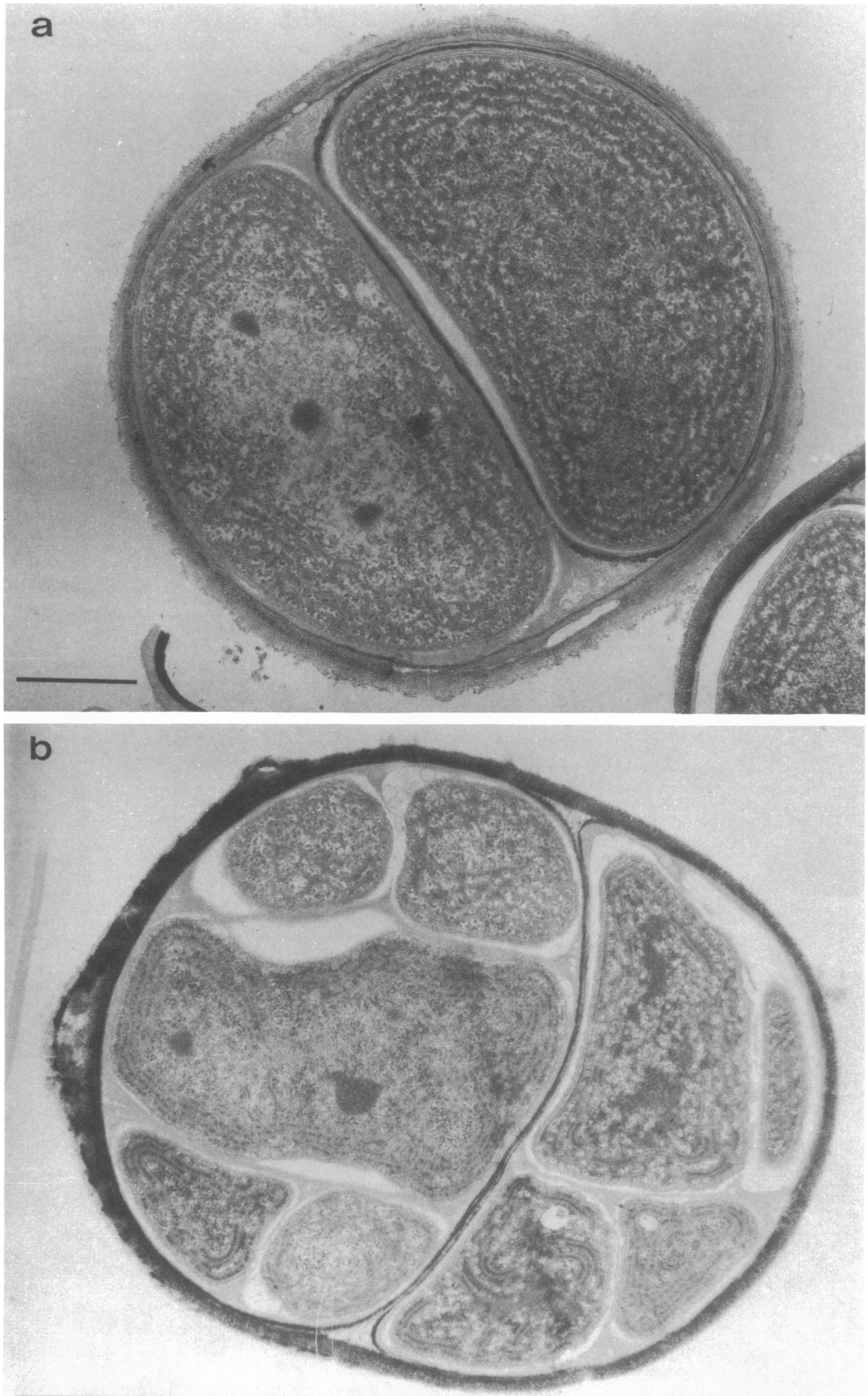

FIG. 27. *Electron micrographs of thin sections of Chroococcidiopsis (7436) grown on a plate of medium BG-11. (a) Two vegetative cells, produced by binary fission. (b) Pair of cells that have undergone multiple fission after one binary fission; note the small size of the cells and the thinness of their individual F layers.*

multiple fission, producing motile baeocytes. The primary character common to all these strains that distinguishes them from *Myxosarcina* is the irregularity of the successive planes of binary fission, even early in vegetative growth.

Semicontinuous observations on development in Cooper dish cultures have shown that the strains of the *Pleurocapsa* group can be divided into two subgroups, which differ in the mode of baeocyte enlargement before the onset of binary fission. In subgroup I, baeocyte enlargement is symmetrical; in subgroup II, it is asymmetrical. The developmental features characteristic of the strains belonging to each subgroup will be separately described below.

Strains of subgroup I. The strains of subgroup I (7310, 7317, 7320, 7440, 7506, 7327, 7322, 7319, and 7324) vary considerably in morphological and developmental complexity, ranging from strains forming small, compact cell aggregates (Fig. 28a) to strains that can produce extensive filamentous outgrowths (Fig. 28b and 29).

The developmental cycle, as observed in Cooper dish culture, of one of the more complex strains (7319) is illustrated in Fig. 29. Symmetrical baeocyte enlargement followed by vegetative divisions in many planes leads to the formation of a small, branched, filamentous aggregate that later develops into a large, irregularly shaped central cluster of cells with peripheral radiating branches. The filamentous habit is maintained by transverse divisions in one plane of the cells at the distal ends of the branches. As the filaments extend, the subapical cells divide either in two planes, to produce dichotomous branches, or in many planes, to produce the large, irregularly shaped clusters of cells from which the filaments radiate. Multiple fission typically occurs in some, but not all, cells of the aggregate, leading to the release of motile baeocytes.

Strains of subgroup II. The baeocytes of strains of subgroup II (7314, 7321, 7516) cannot be distinguished immediately after release from those of strains of subgroup I. The difference first becomes apparent after the baeocytes lose motility and start to enlarge. In strains of subgroup II, enlargement occurs at first by elongation, producing a rod-shaped cell. Very rapidly, however, the enlarging cell broadens at one pole, to become club shaped or pyriform (Fig. 30a and 31). At this stage, the first division occurs, typically in a transverse plane. The planes of subsequent divisions vary; the resulting aggregate is of irregular shape, with a number of filamentous outgrowths. A typical development sequence for a strain (7516) of subgroup II is shown in Fig. 31.

In both subgroups of the *Pleurocapsa* group, as in *Myxosarcina* and *Chroococcidiopsis*, the number of binary fissions that precede the onset of multiple fission is not fixed: the more favorable the growth conditions, the earlier the onset of multiple fission. Indeed, some strains that

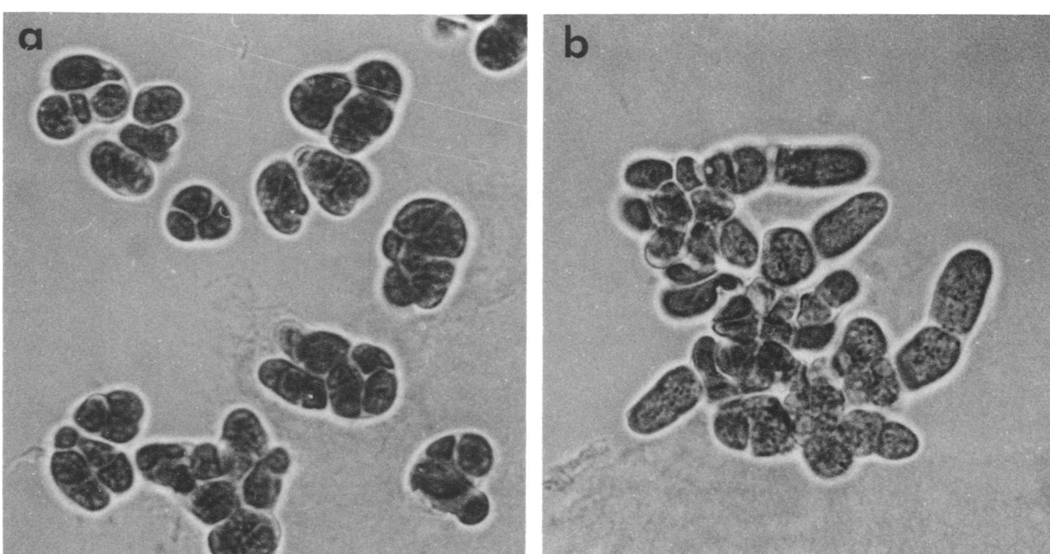

FIG. 28. *Light micrographs of mass cultures of members of the Pleurocapsa group (7317, 7327), grown on plates of media MN and GB-11, respectively. (a) Characteristic compact cell aggregates of strain 7317. (b) Characteristic cell aggregate of strain 7327, showing filamentous outgrowths.* ×1,000.

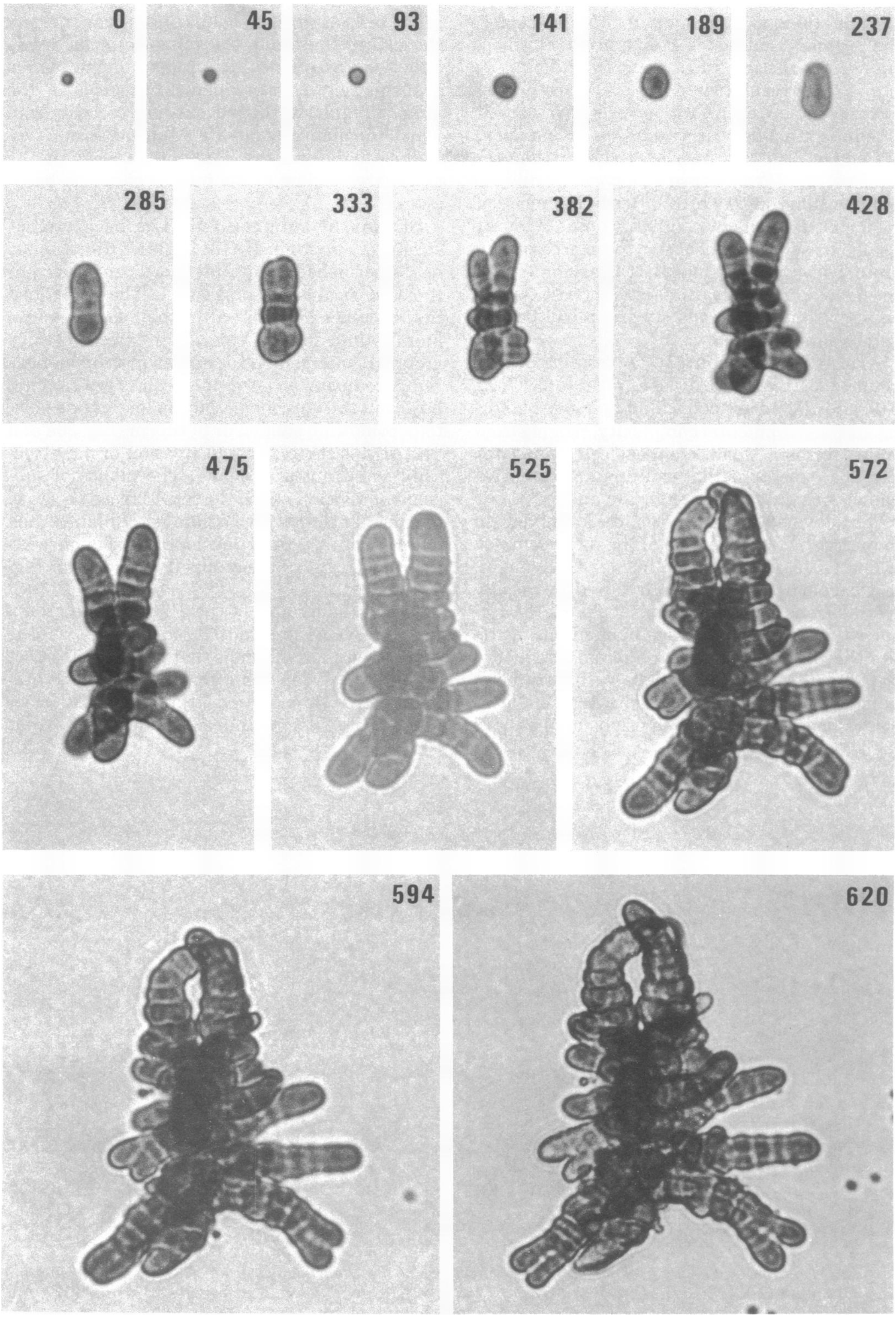

FIG. 29. *Development of a member of the Pleurocapsa group (7319) grown on medium MN in a Cooper dish culture. ×500.*

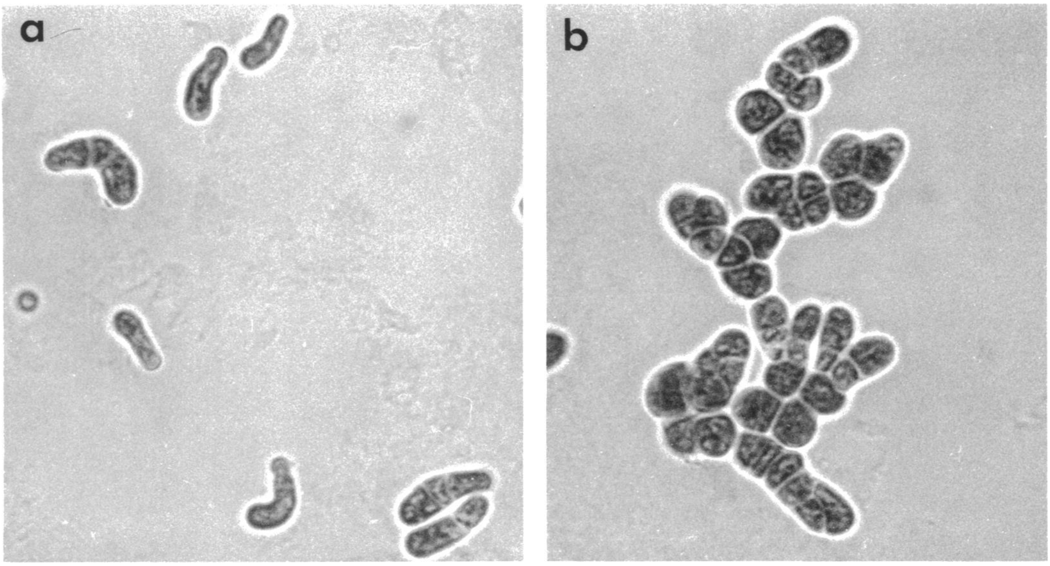

FIG. 30. *Light micrographs of a mass culture of a member of the Pleurocapsa group (7321) grown on an agar plate of medium MN. (a) Asymmetric outgrowth of baeocytes. (b) Characteristic cell aggregates with filamentous appendages.* ×*1,000.*

normally produce filamentous aggregates may, under favorable growth conditions, undergo multiple fission after only one or two binary fissions.

Comparison of Pleurocapsalean Developmental Cycles

Figure 32 presents a schematic summary of the similarities and differences among the seven developmental cycles described in the preceding section.

TAXONOMIC CONSIDERATIONS

Circumscription of the Order *Pleurocapsales*

In many recent taxonomic treatments (3, 6, 13, 14), pleurocapsalean cyanobacteria are classified using the system originally proposed by Geitler (15). He assigned these organisms to two orders, the *Pleurocapsales* and the *Dermocarpales* (synonym, *Chamaesiphonales*), and also included in the latter order the genus *Chamaesiphon*, which reproduces by budding (exospore formation). Table 6 shows the differential characters on which Geitler's ordinal separation is based and lists some of the principal genera that he assigned to each order. Since reproduction by baeocyte formation occurs in members of both orders, the ordinal separation really rests on two differences: members of the *Dermocarpales* are unicellular and display polarity, and members of the *Pleurocapsales* are multicellular and do not display polarity.

Of the genera included in the *Dermocarpales*, *Chamaesiphon* possesses both ordinal properties: it is unicellular, and the cells possess a well-defined apical-basal polarity. Furthermore, it differs from all members of the *Pleurocapsales* in its mode of reproduction. However, none of the other genera now placed in the *Dermocarpales* conforms to the ordinal definition. *Dermocarpa* is unicellular, but the cell is devoid of polarity. *Dermocarpella* displays polarity, but it is not, strictly speaking, unicellular, since its development always leads to the formation of a two- to four-celled aggregate. *Chroococcidiopsis* is not unicellular, always developing as a many-celled aggregate, and does not display polarity; it thus possesses neither of the defining properties of the order *Dermocarpales* but conforms fully to Geitler's definition of the *Pleurocapsales*.

Of the genera included in the *Pleurocapsales*, all lack polarity and all, with one exception, form many-celled aggregates. The exception is *Xenococcus*, a unicellular organism with a developmental cycle like that of *Dermocarpa*.

With the exclusion of *Xenococcus* and the inclusion of *Chroococcidiopsis*, the *Pleurocapsales*, as defined by Geitler, can be justified as a higher taxon. The *Dermocarpales*, on the other hand, remains a heterogeneous assemblage.

A reasonable taxonomic solution is suggested by an analysis of the distribution of five major structural and developmental properties among the genera discussed above (Table 7). *Chamaesiphon* shares, at most, one of these properties

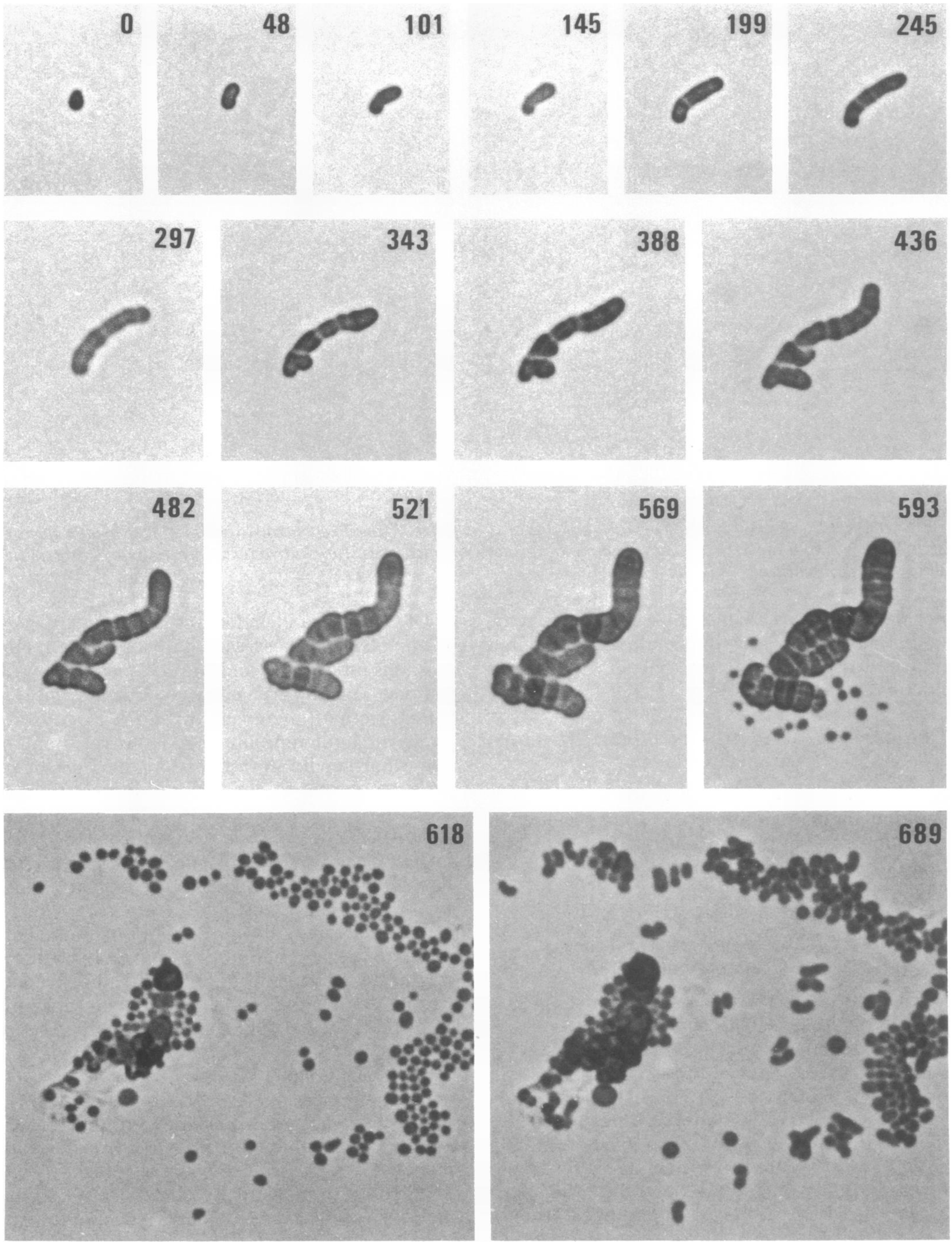

FIG. 31. *Development of a member of the Pleurocapsa group (7516) in a Cooper dish culture. ×500.*

with the other groups. However, the genera *Dermocarpa, Xenococcus, Dermocarpella, Myxosarcina,* and *Chroococcidiopsis,* together with the *Pleurocapsa* group, share at least four of these properties. We therefore conclude that all cyanobacteria that reproduce by baeocyte formation should be placed in a single order, the *Pleurocapsales*, and that *Chamaesiphon* should

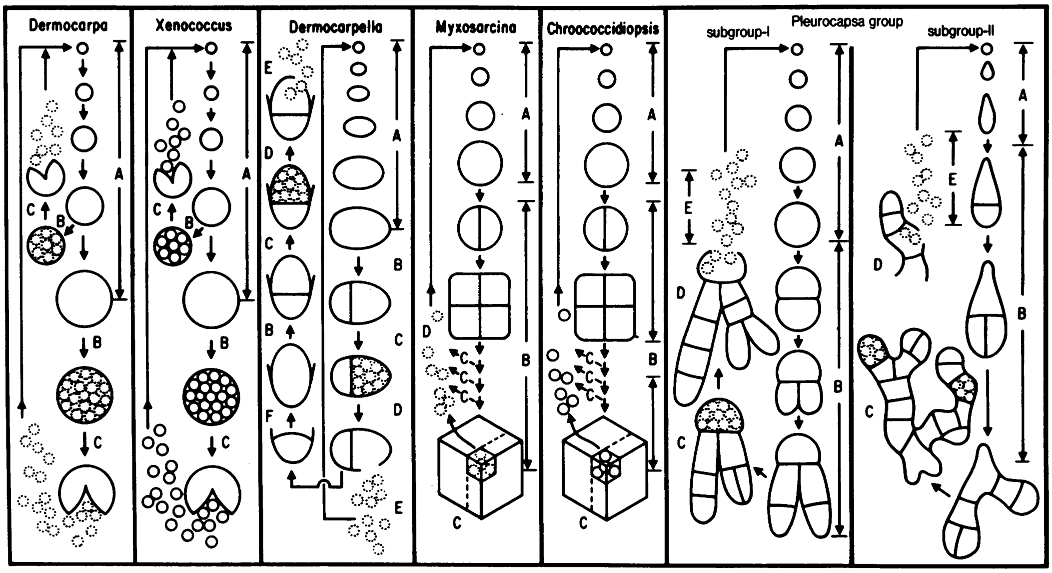

FIG. 32. *Diagrammatic comparison of pleurocapsalean developmental cycles. Baeocytes that are not surrounded by an F layer at the time of release and are, consequently, motile are symbolized by dotted circles to distinguish them from baeocytes that are surrounded by an F layer (solid circles). Dermocarpa: A, symmetric baeocyte enlargment; B, multiple fission, leading to baeocyte formation; C, baeocyte release followed by a brief period of baeocyte motility. Xenococcus: A, symmetric baeocyte enlargement; B, multiple fission leading to baeocyte formation; C, release of immotile baeocytes. Dermocarpella: A, asymmetric baeocyte enlargement; B, binary fission, giving rise to a small basal cell and a larger apical cell; C, multiple fission of the apical cell, leading to baeocyte formation; D, baeocyte release; E, period of baeocyte motility; F, enlargement of basal cell. Myxosarcina: A, symmetric baeocyte enlargement to a predetermined size; B, repeated binary fission in three regular planes; C, multiple fission of almost all the cells in the aggregate, followed by baeocyte release; D, period of baeocyte motility. Chroococcidiopsis: A, symmetric baeocyte enlargement to a predetermined size; B, repeated binary fissions in three regular planes; C, multiple fission of almost all the cells in the aggregate, followed by the release of immotile baeocytes. Pleurocapsa subgroup I: A, symmetric baeocyte enlargement; B, binary fissions in many irregular planes; C, multiple fission of some vegetative cells; D, baeocyte release; E, period of baeocyte motility. Pleurocapsa subgroup II: A, asymmetric baeocyte enlargement; B, binary fissions in many irregular planes; C, multiple fission of some vegetative cells; D, baeocyte release; E, period of baeocyte motility.*

TABLE 6. *Criteria of Geitler (15) for separation of the orders Dermocarpales and Pleurocapsales*

Order	Differential character: Structure	Differential character: Reproduction by means of:	Differential character: Polarity	Major constituent genera
Dermocarpales	Unicellular	Exospores or endospores	Present	*Chamaesiphon* *Dermocarpa* (including *Dermocarpella*) *Chroococcidiopsis*
Pleurocapsales	Multicellular	Endospores	Absent	*Xenococcus* *Myxosarcina* *Pleurocapsa* *Hyella*

be given a different ordinal assignment. A revised definition of the order *Pleurocapsales* follows.

Order *Pleurocapsales*

Cyanobacteria that reproduce by the formation of small, spherical cells (baeocytes) produced through multiple fission of a vegetative cell and released through rupture of the fibrous outer wall of the parental cell. Enlargement of vegetative cells is always accompanied by a progressive thickening of the fibrous outer wall layer. Unicellular members of the order divide exclusively by multiple fission. In other mem-

TABLE 7. *Properties of the principal genera of the Dermocarpales and Pleurocapsales*

Character	*Chamaesiphon*	*Dermocarpa, Xenococcus*	*Dermocarpella*	*Myxosarcina, Chroococcidiopsis, Pleurocapsa group*
Unicellular	+	+	−	−
Reproduction by budding	+	−	−	−
Reproduction by multiple fission	−	+	+	+
Synthesis of F layer surrounding vegetative cells	−	+	+	+
Cell polarity	+	−	+	−
Shared characters:				
Chamaesiphon	5/5	1/5	1/5	0/5
Myxosarcina, *Chroococcidiopsis*, *Pleurocapsa* group	0/5	4/5	4/5	5/5

bers of the order, cell aggregates are produced by binary fission, after which some or all of the cells composing the aggregate undergo multiple fission and release baeocytes.

Redefinition of Pleurocapsalean Genera

Approximately 25 genera that conform to the above ordinal definition have been proposed on the basis of examination of field material. Of these, five can now be recognized and redefined on the basis of differences in structure and development established through studies on pure cultures. Revised definitions of these five genera follow.

Dermocarpa

Unicellular organisms that divide exclusively by multiple fission, leading to formation and release of motile baeocytes. The baeocyte enlarges into a vegetative cell that is intrinsically spherical but may become pyriform or clavate when development takes place under crowded conditions. The successive cleavage stages of multiple fission are not normally detectable by light microscopy.

Xenococcus

Unicellular organisms that divide exclusively by multiple fission, leading to the formation and release of immotile baeocytes. The baeocyte enlarges into a spherical vegetative cell. The successive cleavage stages of multiple fission are readily detectable by light microscopy.

Dermocarpella

Organisms that undergo binary fission to form ovoid aggregates consisting of a large apical cell and from one to three smaller basal cells. Multiple fission of the apical cell then occurs, followed by the release of motile baeocytes. The baeocyte enlarges asymmetrically into an ovoid vegetative cell.

Myxosarcina

Organisms that undergo repeated binary fissions in three planes to form more or less regular cubical aggregates. Multiple fission occurs simultaneously in most cells of the aggregate and is followed by a massive release of motile baeocytes. The baeocyte initiates growth by enlarging symmetrically into a spherical vegetative cell that, just prior to the onset of binary fission, attains a size that is characteristic and constant for any given strain.

Chroococcidiopsis

Organisms that undergo repeated binary fissions in three planes to form more or less regular cubical cell aggregates. Multiple fission occurs simultaneously in most cells of the aggregate and is followed by a massive release of immotile baeocytes. The baeocyte initiates growth by enlarging symmetrically into a spherical vegetative cell that, just before the onset of binary fission, attains a size that is characteristic and constant for any given strain.

Pleurocapsa Group

For reasons previously discussed, it appears premature to assign 12 of the strains examined, all characterized by a similar pattern of development, to an existing genus. Indeed, it is possible that the differences within this group with respect to the mode of baeocyte enlargement may eventually justify the recognition of two genera, distinguished by a character that has no place in the existing classification of pleurocapsalean cyanobacteria. The *Pleurocapsa* group may be provisionally defined as follows.

Organisms that undergo repeated binary fissions in many different planes to produce aggregates that are diverse in size and form. They range from small, compact masses of cells to large structures, consisting of a central mass of cells to which are attached more or less extensive filamentous outgrowths. Multiple fission occurs in some cells of the aggregate and is followed by release of motile baeocytes. The baeocyte may enlarge symmetrically, into a spherical vegetative cell, or asymmetrically, into an elongated vegetative cell, before the onset of binary fission.

Limitations of Field Studies on Cyanobacteria

Descriptions of cyanobacteria by phycologists have been based almost exclusively on examination of field material, either fresh or preserved by fixation or desiccation. The limitations of this approach are particularly serious in the study of pleurocapsalean cyanobacteria. In the first place, these organisms grow very slowly, with the result that observations can rarely, if ever, be prolonged sufficiently to determine the complete developmental cycle. In the second place, different members of the *Pleurocapsales* often grow in close proximity to one another, attached to a common substrate. In the course of the present work, 11 strains of pleurocapsalean cyanobacteria (7310, 7312, 7314, 7317, 7319, 7320, 7321, 7322, 7324, 7325, and 7326) were isolated from a single marine snail shell. They include representatives of three different genera (*Dermocarpella, Myxosarcina,* and *Pleurocapsa*) and probably as many as nine different species. Two adjacent aggregates on such a substrate are, therefore, often composed of members of different species; and the possibility that even a single aggregate may contain more than one species certainly cannot be excluded a priori.

Lastly, observations on field material provide no means of determining the possible extent of environmentally induced variation, which we have shown to be significant in some of these organisms.

All three factors have contributed to the persistence of misunderstandings about the nature of *Dermocarpa, Xenococcus,* and *Dermocarpella,* organisms that were first described in 1858 (5), 1880 (40), and 1907 (29), respectively. Our studies of these organisms in pure culture have confirmed that each genus is characterized by a specific structure and pattern of development and constitutes a readily recognizable taxonomic entity. Yet, Geitler has long rejected the validity of the genus *Dermocarpella* (15, 17), which he synonymizes with *Dermocarpa.* This can be accounted for by his failure to recognize the differences between *Dermocarpa* and *Dermocarpella* with respect to cell division and to polarity, both readily evident in culture but difficult to detect in field material. During multiple fission, *Dermocarpa* sometimes produces relatively large uncleaved segments of cytoplasm. Geitler did not distinguish such division errors (rare events in a *Dermocarpa* population) from the invariable formation of a small basal cell and a large apical cell by binary fission, which is characteristic of *Dermocarpella.* Secondly, he did not distinguish the intrinsic polarity of *Dermocarpella* from the apparent polarity of *Dermocarpa,* which is environmentally induced.

Feldmann and Feldmann (8) recognized and redescribed the distinctive life cycle of *Dermocarpella,* since they were able to observe its development over a considerable period in a mixed algal population maintained in the laboratory. The same authors (8) also report observations on an organism that they interpreted as *Dermocarpa violacea,* the type species of the genus *Dermocarpa.* Their account of this organism contains errors of interpretation that are probably attributable to the fact that the description was based on the examination of groups of cells present on a *Laminaria* frond, unsupported by a developmental study. Feldmann and Feldmann imply, without absolutely committing themselves, that *Dermocarpa violacea* can undergo binary, as well as multiple, fission. Several of their drawings of groups of cells presumed to be *Dermocarpa violacea* are reproduced in Fig. 33. Figure 33a appears to represent a typical *Dermocarpa* group. Figure 33c is probably not; it has the gross appearance of *Xenococcus* undergoing multiple fission, which can easily be misinterpreted as binary fission. The cell group shown in Fig. 33b may have consisted of a mixture of *Dermocarpa* and *Xenococcus.* Since all three populations were assumed by Feldmann and Feldmann to be representative of the same organism, their tentative conclusion that *Dermocarpa* may be able to undergo binary fission is understandable. Ginsburg-Ardré (18) questioned the existence of unicellular cyanobacteria that divide solely by multiple fission. Figure 34 shows her representation of the developmental cycle of *Dermocarpa.* It implies that the developing baeocyte first undergoes repeated binary fissions, to produce a clump of pyriform cells, some of which subsequently undergo multiple

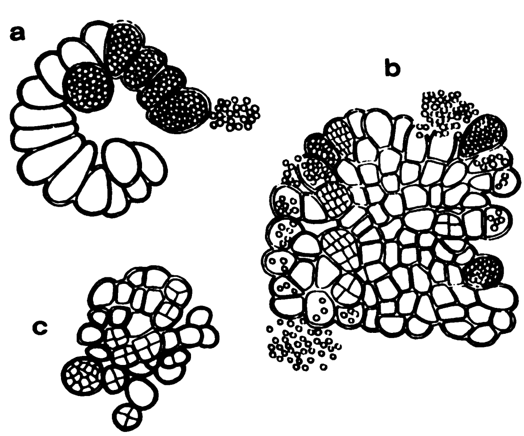

FIG. 33. *Drawing of three groups of cells presumed to be Dermocarpa violacea. Reproduced with permission from Feldmann and Feldmann (Fig. 1 of reference 8).*

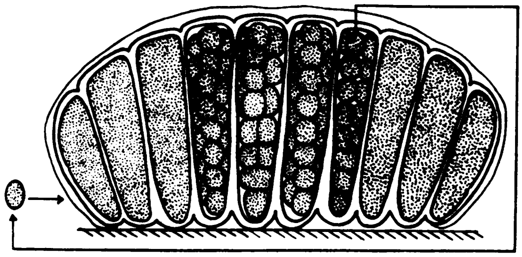

FIG. 34. *Developmental cycle proposed for Dermocarpa. Reproduced with permission from Ginsberg-Ardré (Fig. 1 of reference 18).*

fission. The colony shown in this figure is quite typical of the appearance of a crowded *Dermocarpa* population on a natural substrate; but since Ginsburg-Ardré was unable to study the formation of such colonies, she failed to recognize that each of the individuals of which they are composed arises through the enlargement of a separate baeocyte.

Bourrelly (3) has recently reexamined the 120-year-old dried type material of *Dermocarpa violacea*, type species of the genus, and has concluded that, contrary to the original description, this name was applied to an organism that divides by binary fission. He has therefore proposed changes of generic nomenclature that, if accepted, would be a source of extreme confusion. We believe that old herbarium specimens of cyanobacteria should be left in peace, since interpretations of what they might once have contained are unverifiable. For this reason, we have not adopted Bourrelly's proposals.

Species Identification

Among the 32 pleurocapsalean strains of independent origin that we have characterized, only two clusters show high internal homogeneity in structural and physiological respects: *Dermocarpa* 7437 and 7438 and *Chroococcidiopsis* 7203, 7431, 7432, 7433, 7434, 7436, and 7439. The other strains are sufficiently diverse that each may be a representative of a different species. A satisfactory analysis of identification as species will, therefore, require the assembly of a much larger strain collection.

The ill-defined nature of many existing nomenspecies will be a serious obstacle to detailed taxonomic work. This problem is evident in *Dermocarpa*, where Geitler (14) recognized 28 species, many distinguished by the shapes and the size ranges of the vegetative cells. As we have shown, the cell of *Dermocarpa* is intrinsically spherical but can assume other shapes if growth occurs under crowded conditions; this character is determinatively worthless. The cellular size range for any *Dermocarpa* strain is very large. Its minimum value is determined by the size of the baeocyte; among the six strains that we have studied, two groups can be distinguished in terms of this criterion. The maximum value is determined by the time of onset of multiple fission and can vary widely in any given *Dermocarpa* strain as a function of the specific growth conditions. Hence, size range is, likewise, a determinative character of dubious validity. Few (if any) *Dermocarpa* cultures can be identified through the use of Geitler's key to the species of this genus; and since the type materials are all dead, it is unlikely that other information of determinative value can be derived from their examination.

This nomenclatural problem, by no means confined to *Dermocarpa*, cannot be solved under the provisions of the Botanical Code (38), which now governs the classification of cyanobacteria. The Botanical Code does not recognize cultures as type materials, and nomenclatural types cannot be changed. If cyanobacteria were brought under the Bacteriological Code (26), the problem could, eventually, be resolved through the application of Rule 18h, which permits the replacement of a nonliving type by a culture that corresponds to the original description. Almost any marine strain of *Dermocarpa* could be selected as the new holotype of the type species, *Dermocarpa violacea* Crouan and Crouan 1858, since the original description is very succinct and the type specimen is badly dilapidated. This action would confer a much-needed validity on the generic name, which has been questioned (3), and would provide a point of reference for the recognition of other species. As the foregoing analysis shows, the issue of the nomenclatural code that should govern cyanobacterial classification is not simply a formal matter; it is of great substantive importance, since only the rules of the Bacteriological Code will permit the development of a culture-based taxonomic treatment of the group.

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